Thermal dynamics of bat and insect activity in riparian and woodland habitats

(Australia's only fishing bat, *Myotis macropus*)

Thesis submitted by Alice Barratt in fulfillment of the requirements for the degree of Master of Research

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Statement of Authentication

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution

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Chapter 2

Abstract

Small endothermic mammals have high mass-specific resting energy requirements, making their behavioural and metabolic adaptions to variation in thermal conditions and prey availability critical for survival. My project focuses on the relationships between daily and seasonal variation in thermal conditions and the activity and thermoregulatory energetics of small insectivorous bats and their prey. Since water has greater thermal inertia than air, riparian habitats might provide a more stable thermal environment for insect prey of bats. Consequently, in my first experiment, I investigated whether these relationships differed between riparian and woodland habitat types by sampling bat and insect activity within four replicate sites for each habitat type between December 2018 and August 2019. Bat activity was positively associated with temperature in a curvilinear function, such that bat activity reached a maximum at around 15 °C and tended to decrease on the warmest nights when mean nightly air temperature was above approximately 22 °C. As predicted, not only was bat activity greater overall in riparian habitats, there was also much weaker effect of nightly changes in air temperature. Insect activity was also positively associated with air temperature. Bat and insect activity were positively correlated only in the range of relatively low nightly air temperatures. Bat species differed in their response to temperature. Whereas a significant positive relationship was found for *O. norfolkensis*, *C. gouldii* and *O. ridei*, there was no such relationship for *M. macropus*, *A. australis* and *M. o. oceanensis*. However, the activity of all bats, including the riparian specialist *M. macropus*, decreased during autumn and over winter. In my second experiment, I used miniature temperature sensitive radio-transmitters to measure the body temperature of *M. macropus* in the wild and hence understand whether they relied on torpor as a mechanism to balance their winter energy budget. I found that male and female *M. macropus* used torpor every day during winter, with most bouts lasting just less than 24 h (median duration: 21.3 h) and some multiday bouts lasting a maximum of 144.6 h (6.03 days). Understanding the drivers of activity and the ability to respond appropriately to environmental conditions is a key component in bat and insect biology. My findings highlight the sensitivity of bats and insects to environmental factors and the moderating effects of riparian habitats on nightly thermal conditions. My results also indicate the necessity of long-term sampling for monitoring bat populations because there is strong within site variation in activity levels depending on thermal conditions and season. The new information on torpor use by *M. macropus* changes the perception that torpor is only used extensively during winter

in cold climates. Torpor apparently plays a large role in balancing the tight energy budget of small insectivorous bats even in mild climates. My findings contribute to ecology by providing new insight on the drivers of bat and insect activity and the thermal dynamics of a predator-prey system in different environmental conditions.

Chapter 1: General Introduction

1.1 Energetics as a driver of small mammal ecology

How small mammals manage their energy budget in the face of changes in environmental conditions is a key aspect of their behaviour, ecology and fitness (i.e. survival and reproductive success). For small mammals, energy budgeting is especially important as they have high mass-specific metabolic rates (Nagy, *et al*. 1995) and high additional costs for thermoregulation. This energetic stress is further compounded by an inability to carry substantial fat reserves on a small body frame (Withers 1992). Changes in temperature not only cause large changes in the metabolism of small mammals, but also can strongly affect the availability of their food (Anderson & Jetz 2005; Humphries *et al*. 2005). Food availability can vary widely over short and seasonal time scales. Consequently, these animals have evolved physiological and behavioural mechanisms that enable them to manage their energy budgets, and these traits are of primary importance to increasing their individual fitness and the persistence of their populations.

Field metabolic rate (FMR) describes the average rate of energy expenditure over a short time period (e.g. a day) by free-ranging animals (Nagy *et al*. 1995). Small mammals obviously have a lower FMR than larger ones. However, because the relationship between metabolism and body mass has a scaling exponent of approximately 2/3 (White & Seymour 2003) mass-specific levels of FMR increase exponentially with a decrease in body mass (Nagy 2005). In addition, because smaller bodies have a greater surface area relative to their volume, mass-specific rates of body heat loss to the environment (i.e. thermal conductance) also increase with a decrease in the body size of mammals (Suarez 1992). Resting metabolic rate increased very steeply with decreasing temperature below 30 °C for small (8 to 10 g) bats (Geiser & Bingham 2000). Additional energetic costs, on top of that required for essential body maintenance and thermoregulation, are associated with various behaviours, most substantially because of locomotion (e.g. foraging), and also for tissue growth in juvenile mammals and reproductive output by adults (Brown *et al*. 2004). Therefore, small mammals must forage and consume enough food to meet relatively high daily energy requirements or in excess if accumulating fat reserves (Nagy 2018).

For small insectivorous mammals relying on a temperature-dependant and variable food supply, decreases in temperature will increase their FMR and simultaneously reduce the availability of prey required to meet these higher energy demands. One solution to this

energetic bottleneck is for an animal to increase the effort spent foraging in order to match intake with energy needs, despite the low prey availability. Another option is to attempt to reduce FMR. This can be achieved by avoiding metabolic costs for non-maintenance activities, such as by suppressing reproduction. At a minimum, foraging effort must at least be sufficient for energy intake to match the costs of foraging plus the minimal costs of resting. Energetic costs during rest can be reduced by behavioural mechanisms, for example the selection of warmer roost locations (Turbill 2006a), use of insulated nests or by huddling (Terrien, Perret & Aujard 2011). Basking by small marsupials can reduce their resting energetic costs by 58% when air temperature was 15 °C, and basking behaviour increased when food consumption was reduced (Warnecke, Schleucher & Geiser 2010). The most effective energy-saving physiological mechanism by endothermic animals is the use of torpor, which is defined by a temporary and controlled reduction in body temperature and metabolism. When torpid, animals lower the set-point at which their body temperature is metabolically defended and hence conserve the energy they would have otherwise needed for thermogenesis to maintain a higher normothermic body temperature (Geiser & Ruf 1995; Geiser 2004). Torpor use can reduce resting energy use by up to 99 % compared to normothermic levels (Geiser & Brigham 2000; Geiser 2004) and occurs in a wide range of mostly small birds and mammals (Ruf & Geiser 2015).

The interactions between environmental factors, such as temperature, season and habitat, and the activity of mammalian predators and their insect prey is an important aspect of ecosystem function. Foraging behaviour is thought to be determined by the trade-off between the benefits of energy intake and the costs associated with foraging, which include both energetic costs and increased risk of mortality from predation and other environmental causes (Lima & Dill 1990). Generally, cooling temperature have a contrasting effect on the resting metabolism and activity of endothermic predators compared to their ectothermic prey. Endotherms can remain active at cold temperatures, though in small species at a large energetic cost. Whereas, ectotherms generally do not metabolically defend their body temperature and will become less active at cooler temperatures (Mellanby 1939). However, there are some ectotherm species, such as some moths and bees, that are capable of some thermoregulation (Heinrich 1974). Torpor enables small mammals to conserve energy and hence reduce their need for foraging when their prey is unavailable (Geiser 1995). The energy savings gained by using torpor provide a mechanism for small mammals to be more flexible in how they adjust their foraging behaviour to cope with variation in prey

availability. This makes small insectivorous bats, which use torpor readily (Geiser 2006; Stawski, Willis & Geiser 2014), and their insect prey excellent candidates for studies to investigate the effect of environmental conditions on predator-prey interactions. Furthermore, insectivorous bats are a diverse and ecologically important mammal group that are considered bioindicators of ecosystem health (Stahlschmidt & Brühl 2012; Russo & Jones 2015).

1.2 Small insectivorous bats and temperature-dependant prey

There are approximately 81 species of Australian bats, constituting approximately one quarter of all Australian mammal species (Churchill 2009). Eleven Australian bat species are flying-foxes or smaller fruit and nectar eating bats from the family Pteropodidae, and the remainder are predominantly insectivorous species from six families: Emballonuridae, Megadermatidae, Rhinolophidae, Hipposideridae Vespertiliondae and Molossidae (Churchill 2009). Approximately 60 % of all bat species weigh less than 10 g (Smith *et al.* 2003). Like all small mammals, insectivorous bats have a high mass-specific basal metabolic rate (White & Seymour 2003), and because of their large surface area to volume ratio, a high mass-specific rate of thermal conductance (Geiser 2008). This means that, relative to their size, small insectivorous bats have very high energy costs during resting, especially at temperatures below the lower critical limit of their thermoneutral zone (i.e., < approximately 30 °C) (Speakman *et al*. 2003; Geiser 2006). Considering their high energy requirements and incapability of carrying substantial fat reserves on their small frames, food availability and energy budgeting is a crucial aspect of bat physiology and ecology (Geiser 2006; Geiser & Stawski 2011).

Insectivorous bats have a highly variable food supply because their insect prey are small ectothermic animals. Insect metabolism and activity are strongly dependent on prevailing ambient conditions, with air temperature and humidity being important predictors of activity (Mellanby 1939; Raimondo, Strazanac & Butler 2004). Despite some local adaptation, the activity of flying insects is greatly reduced below approximately 10 °C (Wolbert, Zellner & Whidden 2014). Maximum daily temperature, time of day and the time of year were important predictors of insect activity in a New Zealand forest (Pawson, Marcot, & Woodberry 2017). Bats are typically less active with lower air temperature because foraging is energetically expensive and unproductive, whereas resting and using torpor conserves energy (Wolbert, Zellner & Whidden 2014).

Torpor use plays an integral role in the biology of small insectivorous bats and they show a strong proclivity to enter torpor (Geiser & Brigham 2000; Turbill, Law & Geiser

2003; Geiser & Stawski 2011). Torpor provides substantial energy savings even when ambient temperature is close to the thermal neutral zone for bats. The torpid metabolic rate for Australian Long-eared Bats, *Nyctophilus* was 75 % lower than basal metabolic rate with only a slight decrease in body temperature to 29.3 °C and could be reduced to just 3 % of basal and 0.5 % of resting metabolic rate at the lowest body temperature of 1 to 3 °C (Geiser & Brigham 2000). Furthermore, torpor use by small insectivorous bats during the rest phase is common even during summer in subtropical (Turbill, Law & Geiser 2003) and hot dessert climates (Bondarenco, Körtner & Geiser 2013). During winter or long periods of inclement weather, bats can rely on extended bouts of torpor or hibernation (Turbill 2006b). Because torpid bats have very low costs associated with metabolism and predation (Geiser & Brigham 2012), especially when compared to foraging activity, the decision by bats to attempt foraging is very significant.

1.3 Bat activity and foraging in riparian habitats

A riparian environment provides different conditions and resources than surrounding woodland habitats and these differences might explain why bat activity is often greater in riparian areas (Russo & Jones 2003; Kahnonitch, Lubin & Korine 2018). Firstly, open-water sources are necessary for drinking by flying bats. Secondly, riparian zones tend to have increased humidity relative to other woodland habitats, and, presumably as a consequence, they can also have increased insect activity (Grindal, Morissette & Brigham 1999; Law & Chidel 2002). Furthermore, because water has a specific heat capacity that is 4.2-times greater by mass (and much greater by volume) than air, water has a much greater thermal inertia than air (Portner 2002; Frayne 2013). Riparian habitats therefore provide insects with a more stable thermal environment compared to woodland environments, potentially resulting in a more constant level of riparian insect activity. Additionally, riparian foraging zones are usually less obstructed by dense vegetation than woodland habitat, which makes prey detection and commuting less energetically costly (Almenar *et al*. 2013).

Among the >1300 bat species worldwide (Voigt & Kingston 2016), a small number of species from several different families have evolved the capacity to supplement their insectivorous diet by preying on fish and aquatic crustaceans. Amongst these riparian specialist bat species, there is a spectrum of reliance on piscivory, with some species mostly consuming aquatic-derived invertebrates above the water surface and others eating mostly fish and crustaceans (Aizpurua & Alberdi 2018). Although fish consumption generally offers greater energy density than insects, and possibly other nutritional benefits (e.g. calcium), the

detection, capture and digestion of fish requires specialised foraging behaviours and morphology *(*Aizpurua *et al*. 2014). It has been suggested that the increased flight power of larger bat species, makes them more capable of trawling through water with their hind feet and lifting prey from the water (Aizpurua & Alberdi 2018). Furthermore, larger bat species can take larger prey, which is important as fish are generally larger and heavier than insects (Aizpurua & Alberdi 2018). For example, fish caught by *Myotis capaccinii* were on average 50-times heavier than their typical insect prey (Aizpurua *et al.* 2013). The greater bulldog bat, *Noctilio leporinus*, weighing 65 to 75 g, has the highest incidence of piscivory known among bat species, with approximately 90 % of analysed scats containing fish remnants (Schnitzler *et al*. 1994; Marcelo 2006). Smaller species of fishing bat may be limited in their capacity to capture and consume fish prey and instead rely on smaller aquatic invertebrates.

A recent literature review of all fishing bat species highlighted many gaps in the knowledge of fishing bat diet, behaviour and ecology (Aizpurua & Alberdi 2018). It is not fully understood how fishing bats have evolved as specialist foragers, and how this unusual foraging behaviour influences their energetics and response to temperature and season. Aquatic and riparian ecosystems are an important resource for many species, and it is predicted that fauna will become increasingly reliant on these habitats for refuge from the growing effects of climate change (Selwood, McGeoch & Mac Nally 2015).

1.4 Australia's only fishing bat, *Myotis macropus*

Myotis macropus (8 to 10 g) is a native Australian vespertilionid bat with enlarged hind feet. *Myotis macropus* primarily has a coastal distribution and is rarely found more than 100 km inland, ranging from the Northern Territory along the east coast of Australia through to Victoria (Churchill 2009). The species is listed as Vulnerable in New South Wales in the Biodiversity Conservation Act (2016). *Myotis macropus* is unique among Australian bat species because of its highly specialised riparian foraging strategy. The species uses its disproportionately large hind feet to trawl the surface of waterbodies for prey (Law & Urquhart 2000; Campbell 2009). As a result, *M. macropus* has a very close association with riparian ecosystems (Campbell 2009; Campbell 2011). The species roosts in tree cavities and caves that are very close to water and also commonly in bridge cavities or drainage holes (Dwyer 1970b ; Campbell 2009), with the predominant driver of roost location the proximity to waterways (Campbell 2009). *Myotis macropus* is also unusual among Australian bats because of its harem mating system and, in the more northern parts of its range, multiple reproductive events within a year (Dwyer 1970a).

It is unclear whether foraging in riparian habitats facilitates the ability of *M. macropus* to have multiple breeding seasons each year, with a maternity season that can last from October to March (Dwyer 1970a). The highest nutritional (Ciechanowski *et al*. 2017) and energetic (Speakman *et al*. 2003) requirements for mammals are experienced during pregnancy and lactation. In a recent literature review that classified bat species according to their level of fish consumption, *M. macropus* was classified as 'not confirmed' (Aizpurua & Alberdi 2018). From histological analysis, the diet of *M. macropus* foraging over a freshwater stream in New South Wales (Kerewong State Forest) on the mid-north coast of New South Wales was dominated by invertebrates, with 99 % of prey items represented by aquatic invertebrates and only 1 % represented by fish (Law & Urquhart 2000). However, evidence of fish consumption of free-ranging *M. macropus* has been seen in other studies (Jansen 1987; Robson 1984). The extent to which *M. macropus* consumes fish may be underestimated given histological analysis of faeces from captive individuals fed a diet of fish did not show reveal any signs of piscivory (Robson 1984). *Myotis macropus* may rely on increased piscivory to supplement their nutritional requirements only when invertebrate abundance is low. In the Fish-eating Myotis, *Myotis vivesi*, at a site in the Gulf of California, there was a marginal increase in the consumption of woodland based prey during summer but their prey remained primarily marine fish throughout the year (Otálora-Ardila *et al*. 2013). The Polish Pond Bat, *Myotis dasycneme*, showed no sign of piscivory throughout the year, but an increase in larger insect prey during the lactation period for females (Ciechanowski & Zapart 2012). It is largely unknown what benefits *M. macropus* could gain from specialising in a riparian habitat, nor how this behaviour impacts their response to environmental conditions.

1.5 Preliminary research and project aims

During the course-work year of my Master of Research degree I conducted a preliminary analysis to examine relationships between bat activity and temperature among different bat species using data collected by Gonsalves and Law (2017a). I applied linear mixed effects models to these data, where sampling site was considered a random effect and mean nightly temperature was treated as a main fixed effect. Overall there was a significant difference in the relationship between activity and temperature among the bat species ($p = < 0.001$). For *M. macropus*, there was no significant relationship between activity and temperature ($p = 0.3873$); whereas, a significant positive relationship was found for other commonly recorded bat species: *Chalinolobus gouldii* ($p = < 0.001$) and *Mormopterus ridei*

(p = 0.022). However, since data from Gonsalves and Law (2017) was collected over just a few sampling nights (n = 2 to 3) at each site and only within the warmer seasons, it remained unclear how these relationships might be affected by a larger range of nightly thermal conditions.

This Master of Research thesis aims to gain a better understanding of how environmental conditions influence the foraging activity and resting energetics of small insectivorous mammals. To do so, two data chapters (chapters two and three of the thesis) were designed to examine different, yet interlocking, ideas. The first data chapter, entitled 'Thermal and seasonal effects on bat and insect activity in riparian and woodland habitats', investigated the drivers of daily and seasonal bat activity in different habitats. Bat and insect activity were sampled in riparian and woodland habitats, both intensively and long-term, so the influence of daily temperature and season on activity could be compared between habitat types and species. The experimental design of this chapter allowed us to estimate in detail the influence of environmental conditions on the within-site variability of bat and insect activity.

The second data chapter, entitled 'Torpor and activity during winter by Australian fishing bats, *Myotis macropus*' determined the use of torpor, winter energetics and activity patterns of Australia's only fishing bat, *Myotis macropus*, by radio tracking with temperature sensitive transmitters. *M. macropus* are uniquely tied to riparian habitats and could benefit energetically from a more consistent and abundant insect availability (due to the thermal inertia of water), reduced energetic costs of commuting to foraging habitat and constant water availability. The seasonal activity rates of *M. macropus* will be analysed in the first chapter. This second chapter will address the behaviour of individual *M. macropus* when resting. Torpor use has not been studied in this species so this chapter will provide the first information on the resting energetics *of M. macropus* and allows insight into the energy budgeting of bats in riparian ecosystems when food availability is at its lowest.

All research was authorised by the Western Sydney University, Animal Care and Ethics Committee (ACEC) (project: A13008) and carried out under a NSW scientific licence (SL101936) issued under the *Biodiversity Conservation Act 2016* by the NSW National Parks and Wildlife Service.

Chapter 2: Bat and insect activity in riparian and woodland habitats: effects of season and air temperature

2.1 Introduction

Understanding how animals adjust their activity in relation to short-term and seasonal changes in environmental conditions is an important aspect of ecology and the conservation management of wildlife. As activity come with inherent costs in terms of both energy expenditure and increased mortality risk from predation and other environmental causes (Lima & Dill 1990), animals rely on environmental conditions to predict the likely success of foraging activity. Season can be good predictor of environmental conditions and hence most animals respond to changes in photoperiod as an indicator of expected environmental conditions (Gwinner 1986). However, environmental conditions can also vary dramatically among days and even shorter time periods, especially in temperate climates. Daily and seasonal changes in environmental conditions influence the costs and benefits associated with activity, at times creating difficult conditions for animals. Responding appropriately to environmental factors is a fundamental component of how animals survive and successfully reproduce, and hence understanding these relationships is a key objective of ecology. Furthermore, how animals regulate their behaviour in response to changing environments is also necessary if we are to reliably survey populations of free ranging animals and manage ecosystems to promote wildlife conservation.

Environmental conditions, especially temperature, are particularly important for small endothermic animals like bats, which require high rates of food intake relative to their body mass. With a decrease in body mass, the mass-specific resting metabolic rate of mammals increases exponentially (Schmidt-Nielsen & Knut 1984; Suarez 1992), and thermal conductance also increases because of a higher surface area to volume ratio (Chew, Lindberg & Hayden 1967; Withers 1992). A high mass-specific metabolic rate that further increases rapidly with a decrease in air temperature below the thermal neutral zone is problematic for small insectivorous bats as the availability of their prey activity also decreases at a colder air temperature (Mellanby 1939; Raimondo, Strazanac & Butler 2004). Because they are ectotherms, the principle source of energy to maintain body temperature for insects is the environment (Clarke 2017). Some insect species are capable of a limited form of endothermy (Heinrich 1974). However, there is generally a strong positive correlation between insect activity and air temperature (Heinrich 1995), until air temperature exceeds a maximum

critical temperature, which can vary depending on species and location (Curtis *et al*. 2008). The decrease in insect abundance at cooler temperatures means that insectivorous bats must cope with the problem of increased metabolic demands with decreased food availability.

Bats and insects provide a good system to study the influence of energy costs and thermal conditions on predator prey interactions. Bats are ecologically and economically important because they are generally opportunistic foragers, eating a range of insect orders (Gonsalves *et al*. 2013a; Milne, Burwell & Pavey 2016; Vestjens & Hall 1977), and are capable of consuming 61 to 84 % of their own body mass in a single night (Kalka & Kalko 2006). It was estimated that, by consuming pest insects, bats prevent $22.9 billion worth of crop loss per annum in the United States alone (Boyles *et al*. 2011). Additionally, bats can reduce the numbers of disease-carrying insects like mosquitoes (Reiskind & Wund 2009) and flies (Boyles *et al*. 2011). Bat activity is typically positively associated with temperature (Bender & Hartman 2015; O'Donnell 2000; Russo & Jones 2003) because although low temperatures would increase their resting energy requirements, many bats cope with this problem by using torpor to temporarily reduce their metabolism and body temperature and hence conserve energy (Turbill, Law & Geiser 2003; Geiser & Stawski 2011). Thus, small insectivorous bats have very high energetic costs when they are active and often very low energy costs when resting because they often enter into torpor. The big difference in energy use between these options increases the consequence of decisions about when to foraging as a function of environmental variation in conditions. Bats are also an important mammal group to study activity patterns because they can be readily quantified using an index based on echolocation calls (Anderson, Law & Tidemann 2006;Wolbert, Zellner & Whidden 2014; Law *et al*. 2015). Therefore, insectivorous bats are a good model group for studying the environmental drivers of small mammal activity.

The activity of small insectivorous bats in seasonal climates follows a predictable fluctuation throughout the year, which is usually cued by changes in the photoperiod (Usman, Subbaraj & Subramanian 1990). Reproduction and growth are scheduled to take place in the productive season during spring or summer (Parsons, Jones & Greenaway 2003; Agosta *et al*. 2005; Johnson *et al*. 2017) when there are resources available to support the added energetic demands (Speakman *et al*. 2003). Whereas, activity is reduced during the less productive winter season, when bats in cold temperature climates are known to hibernate (Halsall, Boyles & Whitaker 2012). Small insectivorous bats, especially from the Vespertilionidae family, show a strong proclivity to enter torpor, with short bouts during the rest phase

common even during summer in subtropical areas (Turbill, Law & Geiser 2003). Season also has an impact on the activity of the insect prey of bats (Wolda 1978; Wickramasinghe *et al*. 2004; Pawson, Marcot & Woodberry 2017), with a decline in activity typically recorded during winter (Johnson *et al*. 2017). Even in tropical habitats insect abundance can fluctuate throughout the year (Denlinger 1980; Lowman 1982). However, although season is a predictor of activity of insects and bats, to what degree this effect is simply a consequence of daily changes in conditions, and how the effects of season and daily conditions might vary among different habitat types, is not fully understood.

Bat activity can vary widely over a short time period because of a range of factors like daily weather and prey availability. The activity of both bats and their insect prey are positively associated with air temperature and not surprisingly a number of studies have found a strong correlation between insect abundance and bat activity (Hayes 1997; Fukui *et al*. 2006; Wolbert, Zellner & Whidden 2014). Bats modify foraging behaviours in response to changes in insect activity associated with weather conditions and habitat preferences (Wojciechowski *et al*. 2007; Gonsalves *et al*. 2013a). Air temperature at dusk is known to strongly influence nightly bat activity (O'Donnell 2000; Schwab & Mabee 2014; Bender & Hartman 2015). However, torpor may not be induced in a direct response to air temperature, but rather to manage low prey availability indicated by low air temperature (Wojciechowski *et al*. 2007). Insectivorous bat activity generally begins to increase above 10°C, which reflects the temperature threshold of flying insect in a mild climate (Rydell, Entwistle & Racey 1996; Threlfall, Law & Banks 2012). This suggests that temperature is used as a signal of food availability and insect abundance is driving bat activity. However, this predator prey interaction is not fully understood, and more information is needed to understand how these relations are influenced by environmental conditions.

The short- and long-term environmental drivers of bat foraging activity might vary depending on habitat type. Bat activity is influenced by habitat type and vegetation structure (Threlfall, Law & Banks 2012), with habitat preferences varying by species depending on echolocation design and wing morphology (Norberg 1994). The activity of different bat species has been associated with water sources and other aspects of landscape heterogeneity (Grindal, Morissette & Brigham 1999; Kahnonitch, Lubin & Korine 2018; Law & Chidel 2002; Russo & Jones 2003). Lakes and rivers have been found to have five times higher activity and 14-times greater foraging activity compared to dry habitats (Blakey *et al*. 2017). Increased bat activity in riparian zones could be attributed to lowered energetic costs of

echolocation and commuting in an open area (Rainho, Augusto & Palmeirim 2010), increased humidity and resources providing a higher abundance of insect prey (Raimondo, Strazanac & Butler 2004) and the need for drinking over water. Furthermore, because water has greater thermal inertia than air (Frayne 2013; Portner 2002), riparian habitats might provide a more constant thermal environment for the insect prey of bats. Thus, bats foraging in riparian habitats might find available prey, and consequently remain active, when bats foraging in other habitats may not. The Large-footed Myotis, Myotis macropus, is the only Australian bat species restricted exclusively to a riparian ecosystem and has a diet that is predominantly aquatic-based (Law & Urquhart 2000; Campbell 2009). When investigating the distribution of *M. macropus* throughout the Sydney estuary during summer and autumn there was no relationship between daily minimum temperature and detected nightly activity (Gonsalves & Law 2017a). Similarly, in a study conducted in the United States, the number of nightly bat passes over streams showed no correlation to air temperature (Seidman & Zabel 2001). Riparian habitats have known benefits for bats and insects, which might decrease the impact of daily weather variations on bat activity.

In this chapter I undertook an experiment designed to quantify the effects of daily changes in weather conditions and longer-term seasonal patterns on the activity of insectivorous bats and their flying insect prey at riparian and woodland habitats. An index of bat and insect activity was measured at four sites in riparian habitat along the Nepean river and four sites in nearby woodland over a period of nine months. The aim of this study was to assess how environmental factors, primarily ambient air temperature, season and habitat, affect the activity of both bats and their insect prey. Furthermore, the study assessed how insect prey activity influences bats in riparian and woodland habitats.

I predicted that bat and insect activity will respond to daily variation in thermal and other weather conditions and independently to the time of year, i.e. a seasonal effect, presumably caused by detection of the changing photoperiod. Furthermore, I suggest a daily thermal effect will be weaker in the riparian compared to woodland habitat because the thermal inertia of water bodies should dampen daily fluctuations in thermal conditions for insects living in and close to water. However, a seasonal effect should still be apparent for both habitats. Finally, I predict a positive correlation between bat and insect activity because bats will alter foraging activity to match prey availability, thus maximising foraging success and reducing unnecessary costs.

2.2 Methods

Study area

The study was conducted along a 20 km stretch of the Nepean River and in adjacent remnant patches of woodland between Penrith (-33.723, 150.683) and Windsor (-33.605, 150.817), approximately 50 km west of the Sydney CBD, New South Wales, Australia. The local area has a humid, subtropical climate, with average daily minimum and maximum temperatures ranging from 3.5 to 17.7 °C in July (winter) and 17.9 to 30. 4°C in January (summer) at the nearby Richmond RAAF base weather station (1993 to current; Australian Bureau of Meteorology). Systematic sampling of bat and insect activity was undertaken across four replicate sites for each of two habitat types (riparian and woodland) between December 2018 and August 2019. At each site, bat calls were recorded from sunset to sunrise over a two-week period each month, with acoustic recorders rotated among 8 sites representing two treatments; four riparian and four woodland sites. Insect activity was surveyed for two nights each month on nights when bat sampling was not undertaken.

All sites were at least 2 km apart and were within a 100 km^2 area. Sites in riparian habitat were a located along the Nepean River and the nearby lakes at the Penrith White Water Stadium. Detectors were placed on the shore, parallel to the water's surface to increase the likelihood of detection for *M. macropus* and other species foraging and commuting over water (Gonsalves & Law 2017a). Riparian sites were at least 500 m from known roosting colonies. Sites in woodland habitats were located a minimum distance of 1.5 km from the river in two nature reserves (Agnes Banks and Wianamatta) and on remnant vegetation on the Hawkesbury Campus of Western Sydney University. Woodland areas ranged between 1.4 km^2 and 6.3 km^2 and were surrounded by a rural or semi-urban landscape. In woodland sites, detectors were locked to trees approximately 2 m from the ground, and with microphones directed along a fire trail (track), as forest tracks are typically used by commuting bats (Law & Chidel 2002).

Local external environmental conditions (air temperature, wind speed and rainfall) were taken from automated weather stations at the site of the EucFACE experiment (Ellsworth *et al.* 2017), which is located at the Hawkesbury Institute for the Environment, Western Sydney University Hawkesbury campus (-33.617, 150.740). This site was < 12 km from all sampling sites.

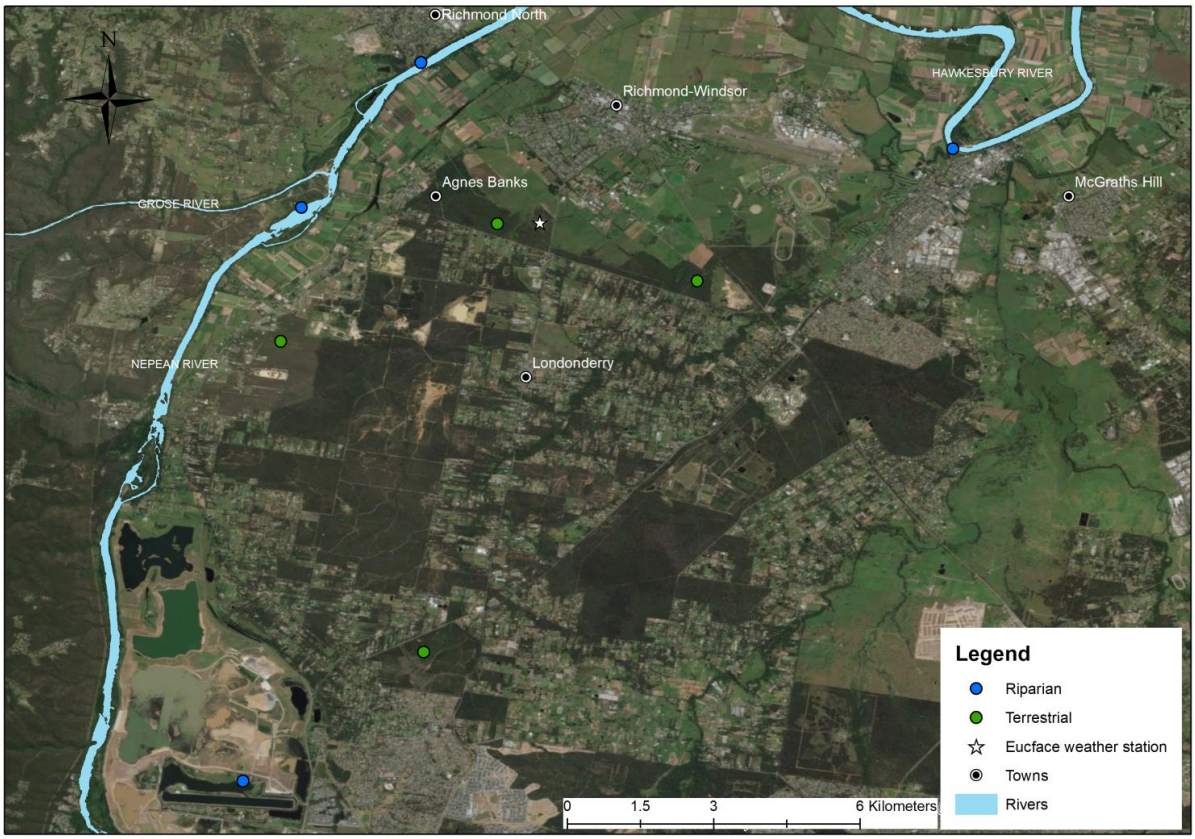

Figure 1: Map of the study area showing the location of sites in riparian (blue dots) and woodland (green dots) habitat. The location of the weather records is indicated by a star symbol.

Bat activity

Bat calls were recorded using passive acoustic (ultrasonic) detectors (SMZC, Wildlife Acoustics). Using bat calls recorded by acoustic detectors as an index of bat activity is a well-established and recognised practise in bat ecology (Anderson, Law & Tidemann 2006; Wolbert, Zellner & Whidden 2014). This method is capable of detecting patterns and changes in bat activity (Law *et al.* 2015). Detectors were programmed to record from sunset to sunrise each night for two weeks of each month of sampling. However, rechargeable batteries used over the course of the study did not retain their charge and some detectors lost power before the end of the two-week sampling period in the last two months of sampling, allowing for only 8 nights of data in each of these two months. Detectors recorded calls as zero crossing sound files, that lasted a maximum of 15 seconds.

The zero crossing sound files recorded by the acoustic detectors were batch-processed using an automated call identification software program called AnaScheme (Gibson & Lumsden 2003). The program used a key developed for bat fauna of coastal Sydney (Unpublished data – B. Law). Though the accuracy of AnaScheme with the coastal Sydney

key has not been formally tested, an assessment of a key for a different region reported that 91 to 99 % of files were identified correctly (Adams, Law & Gibson 2010). Files that AnaScheme classified as "noise" or that had insufficient data for identification as a bat call were excluded from all analyses. Sound files that were positively identified as a bat call but with <50 % of pulses that could be attributed to one species were grouped together as unknown species but included in counts of total bat activity in analyses.

The AnaScheme key identification criteria cannot distinguish between the calls of *Myotis macropus* and two species of co-occurring *Nyctophilus* as they share similar call characteristics of steep, linear pulses, often with a central kink (Pennay, Law & Reinhold 2004). The identification key assigned these type of calls with steep linear pulses as *Nyctophilus* spp., however the calls of *M. macropus* and *Nyctophilus* can be distinguished manually using particular call characteristics (Table 1).

Table 1: Defining call characteristics of *Myotis macropus* and *Nyctophilus* species from Bat calls of New South Wales (Pennay, Law & Reinhold, 2004)

Myotis macropus	***Nyctophilus* species group**
Pulse interval < 75 ms	Pulse interval < 95 ms
Initial slope of call > 400 OPS	Initial slope of call < 300 OPS
Starting between 70-80 kHz - dropping to between 35-40kHz	Starting between 70-108 kHz - dropping to between 47-55 kHz
Central kink in slope, second part of call having lesser slope that the first	Can have two changes in the slope, in the middle or lower half.
Longer call sequences	Shorter call sequences

A colony of *M. macropus* roost in the Yarramundi and Richmond Bridges along the Nepean river, hence there was a high level of *M. macropus* activity at riparian sites given their proximity to these roost sites. Since a large number of steep linear calls were recorded in riparian sites (total = 32,040 calls), it was not feasible to manually assess each call. *Myotis macropus* only forages over waterways and roost sites are close to water (Campbell 2009; Campbell 2011) whereas *Nyctophilus* spp. primarily forage close to vegetation (Lumsden, Bennett & Silins 2002; Threlfall, Law & Banks 2013). Therefore, all linear-type bat calls identified by AnaScheme in woodland sites were assigned to the *Nyctophilus* spp. group, whereas these calls recorded at riparian sites were classified as *M. macropus*. To corroborate this assumption, a randomly selected 5 % (5 % or a minimum of ten calls were identified, or all recorded calls were manually verified if total number of calls was < 10) subset of linear-

type calls recorded from all sites in every two-week period were manually classified as *M. macropus*, *Nyctophilus* spp. or unknown based on the criteria shown in Table 1. At riparian sites, 92 % of the 1601 linear calls analysed manually were confirmed as *M. macropus* and 3.7 % were identified as probable *Nyctophilus* spp.. At woodland sites, 92 % of the 246 manually analysed linear calls were identified as *Nyctophilus* spp. and only 0.9 % had characteristics indicative of *M. macropus*. I accepted this level of error and assigned linear calls to *M. macropus* or *Nyctophilus* spp.. on the basis of the habitat in which they were recorded.

Insect mass and count as an index of activity

UV-light traps (light-activated; Australian Entomological Suppliers) were used to sample the activity of flying insects over two nights each month at each site (Lowman 1982). Light trapping was undertaken on nights without bat sampling as lights may have disrupted normal bat activity in the area (Aurelie *et al*. 2014; Pauwels *et al*. 2019). The insect sampling night was assigned randomly, regardless of the weather prediction, to four sites (two woodland and two riparian) on the same night. Samples were collected in the morning and then stored at -30 °C, until they were thawed for processing (Knapp 2012).

After removal from storage, the wet mass of each insect sample was measured, and a random 25 % of the wet mass was separated as sub-sample for sorting. After this, the subsample, or whole sample if wet mass was < 3 g, was spread across a 0.5 cm grid and sorted into four size classes (1: < 5mm; 2: 5 to10 mm; 3: > 10 to 15 mm; and 4: > 15mm) and identified to three order-level groups (Lepidoptera, Diptera, Coleoptera and all others) using a dichotomous key (Castner 2000). Each of the 16 combinations of size and taxon was counted and placed into a brown paper bag for drying in an oven for 48 h at 70 °C. The mass of each dried insect sample was measured at a precision of 0.0001 g.

Data analysis

Nightly weather data was the average of measurements from 6 pm to 6 am. The mean nightly temperature and rainfall for the entire study period was plotted to show the seasonal variation in weather throughout the study. The total number of bat call files recorded during each night was used as an index of nightly bat activity for each site. Hence forth, bat activity will refer to the number of bat calls recorded per night. The average bat activity per night, each month was calculated for every site. This was used to describe total bat activity and variation among sites within habitat types and displayed in a column graph.

Data analyses were conducted using the software *R* version 3.4.2 (R Development Core Team 2009) as implemented by the software RStudio version 1.1.423 (RStudio Team 2015). Mixed effects (LME) models were fitted by restricted maximum likelihood method to test the fit of various models explaining variation in nightly bat or insect activity using the function lmer in the R package lme4 (Bates & Maechler 2009). Site was included in models as random effect on the intercept to account for repeated measures and differences in average activity among the replicate sites. Significance of fixed effects was tested using *p*-values and degrees of freedom for the t-test based on the Satterthwaite approximation as implemented by the lmerTest package (Kuznetsova *et al*. 2017). Models were simplified from an initial global model by the sequential removal of non-significant fixed effects, starting with least significant interactions. Partial effects plots, which display the model-predicted mean effect, confidence intervals and partial residuals associated with a single variable statistically independent of the mean effects of other variables included in the same model, were derived using the function visreg in the package of the same name (Breheny & Burchett 2017).

Relationships between insect activity and nightly weather variables (mean air temperature, wind speed and rainfall) were assessed using LMEs. Response variables for insect activity were mass (g) per night and number of insects per night according to insect order, and mass (g) per night and number of insects per night according to size class. LME models for each response variable were fitted as a function of air temperature, with an additive effect of wind and rain. Rainfall was included in final insect models, and wind was not. Rainfall was not significant in all models but for consistency was included in all models.

Total bat activity was fitted as function of insect mass/count, with mean nightly air temperature included as an interaction, while habitat type and air temperature were included as an additive fixed effect (noting that bats and insects were never sampled together on the same day at a given site). Season, rainfall and wind were tested in the global model but removed as they were insignificant. Insect data were pooled among insect orders and size classes to provide a measure of total insect activity, by mass (g) and count. Then the mean nightly activity of bats and insects in riparian and woodland sites were calculated and matched according to date.

2.3 Results

Activity of all bat species

Mean nightly air temperature ranged from 5.0 °C to 28.9 °C during the study period with highest temperatures occurring in late January to February and lowest in June and July (Figure 2). Over the night, mean hourly rainfall ranged from 0 to 2.1 mm and mean wind speed from 0.5 to 3.0 m/s during the study period (Figure 2).

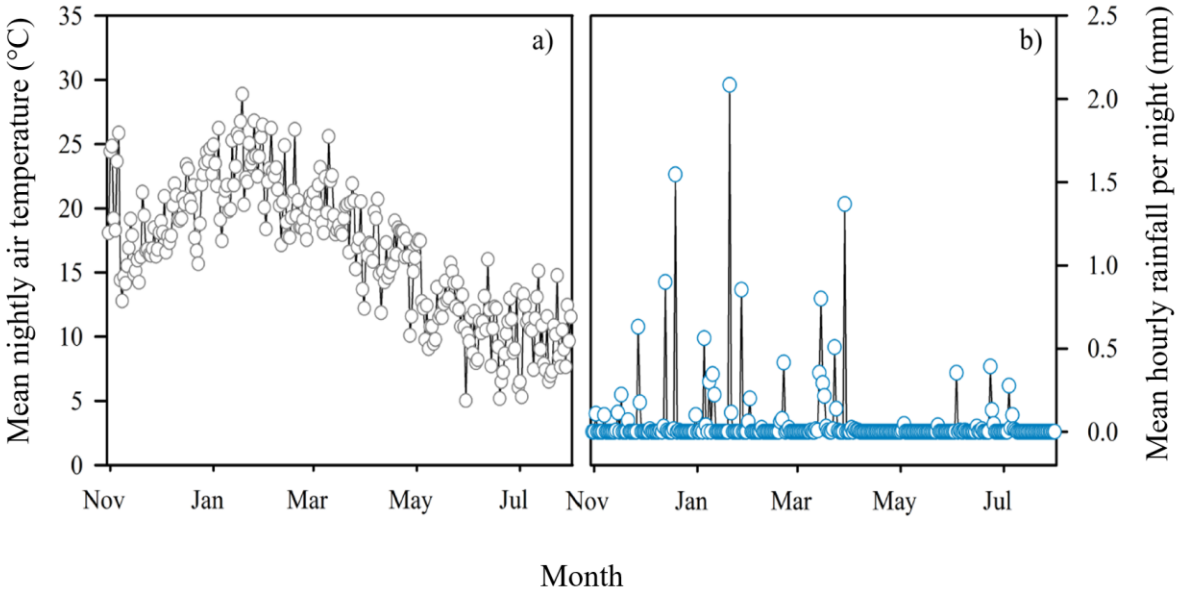

Figure 2. Air temperature averaged over the hours (6 pm to 6 am) of each night (a) and rainfall averaged over each hour during the night (b) over the duration of the study**.**

A total of 96,198 sound files identified as bat activity were recorded, and 67,315 of these files (70 %) were identified to species level. Bat activity was greater in riparian sites compared to woodland sites (Figure 3). Bat activity was generally higher from December to April and then decreased from April to July, with variation in bat activity greatest among nights in April and May.

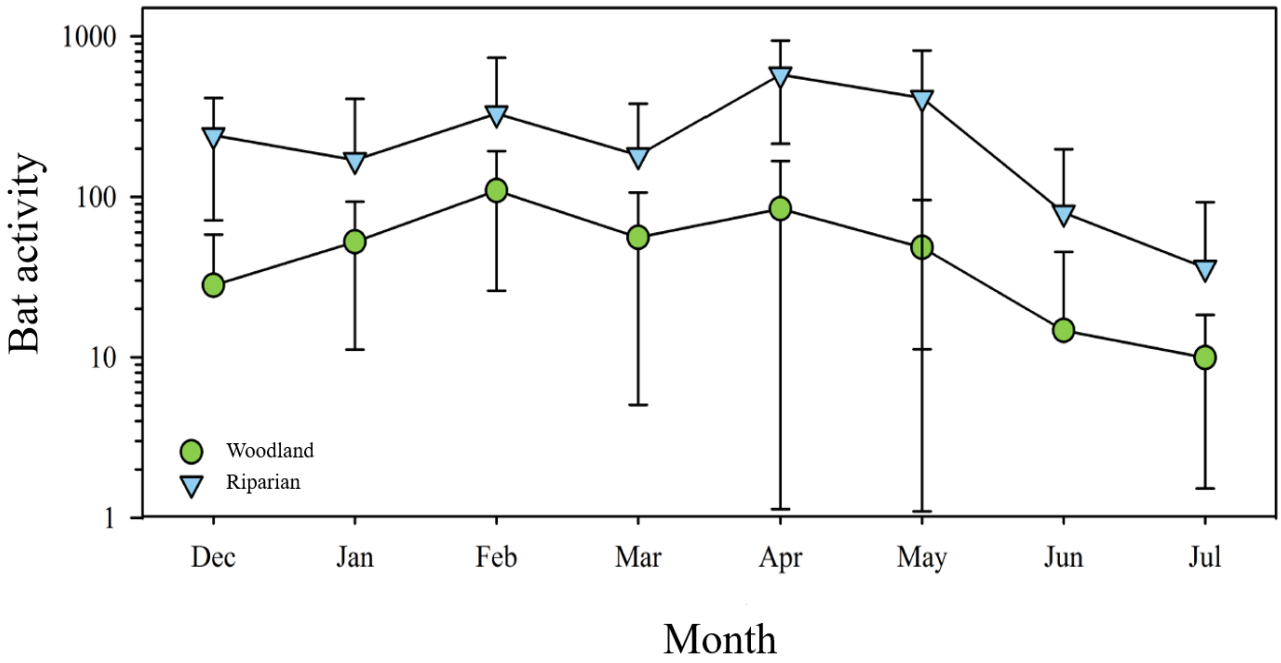

Figure 3. Average bat activity per night from the four sites (± SD) for the riparian and woodland habitat types. Bat activity is displayed on a $\log_{10}$ scale.

Bat activity in both habitats appeared to increase on warmer nights up until mean nightly air temperature reached around 10 to 15 °C, and maximum number of calls recorded per night at any given temperature was much higher in the riparian (Figure 4).

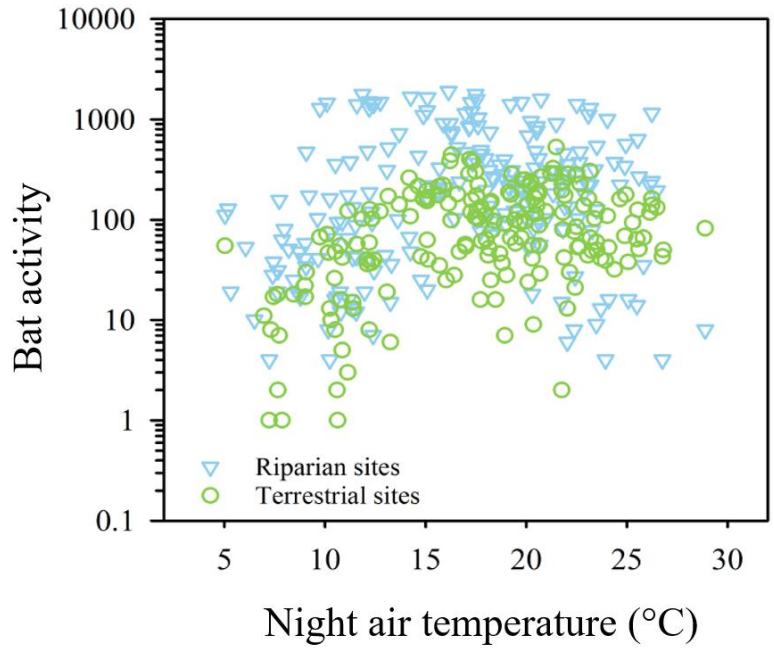

Figure 4. The sum of bat activity per night among the four sites for the riparian versus woodland habitat types plotted as a function of the mean nightly air temperature (°C). Bat activity is displayed on a $\log_{10}$ scale.

The most parsimonious linear mixed effects model fitted to explain nightly variability in bat activity included a significantly positive effect of air temperature fitted as a quadratic function (Table 2, Figure 5). Bat activity increased with increasing nightly air temperature but began to plateau around 20 °C. The interaction between habitat type and air temperature indicated a weaker effect of increasing air temperature on bat activity in the riparian habitat, and that, the positive effect of riparian compared to woodland habitat type on bat activity was greatest at lower minimum air temperatures. Day of the year affected bat activity according to a sinusoidal curve, and the interaction between habitat type and day of the year indicated a stronger reduction in bat activity during the winter season in the woodland compared to the riparian habitat. The most parsimonious model also retained a significant but weak negative effect of wind speed.

Table 2. Results of a linear mixed effects model fitted to explain the variation in nightly bat activity (log_{10}). Site was treated as a random effect on the intercept. The initial global model included the following non-significant fixed effects that were removed sequentially to derive the final model: Rainfall, Rainfall^2 and Wind^2.

Fixed effect	**Coeff**	**SE**	**DF**	**t-value**	***p*-value**
(Intercept)	0.135	0.3	84.0	0.5	0.639
Air temperature (°C)	0.117	0.0	647.6	4.3	<0.001
Habitat (riparian)	0.762	0.3	16.3	2.9	0.011
Air temperature^2 (°C)	-0.003	0.0	647.7	-3.6	<0.001
Wind (m/s)	-0.127	0.1	647.2	-2.3	0.019
Day of year (DOY)	0.000	0.0	648.8	0.3	0.801
Sin(2π/365 × DOY)	0.524	0.1	647.6	8.5	<0.001
Cos(2π/365 × DOY)	0.153	0.1	647.4	2.8	0.006
Air temperature (°C) × Habitat type (riparian)	-0.019	0.0	647.4	-2.4	0.015
Habitat type (riparian) × DOY	0.001	0.0	650.3	2.9	0.004

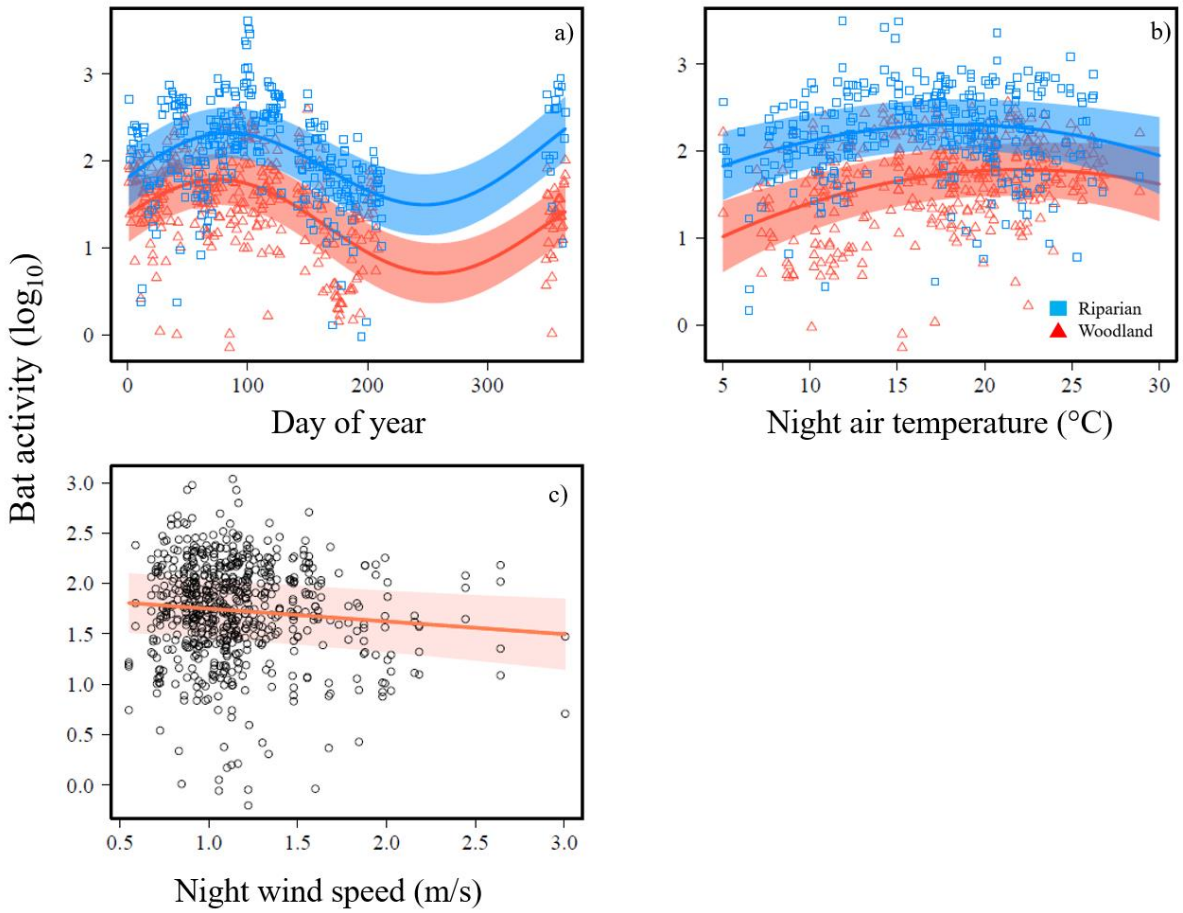

Figure 5. Partial effects plots from the linear mixed effect model fitted to explain nightly variation in bat activity (log_{10}), as a function of a) day of year (season), b) night air temperature (°C), c) night wind speed. Partial effects plots show the mean estimated effect of each explanatory variable that is statistically independent of other fixed effects included in the model. For all effects see model results shown in Table 2.

Activity of different bat species

Calls were identified from 18 bat species, with *Myotis macropus* the most commonly recorded species (47 % of all calls; Figure 6). All species were recorded in both riparian and woodland habitats, apart from *Saccolaimus flaviventris* which was only recorded in riparian habitats. Linear-type calls were attributed to *M. macropus* and *Nyctophilus* species in riparian and woodland habitats, respectively, and manual identification of a subsample of these calls confirmed this assumption (see Methods).

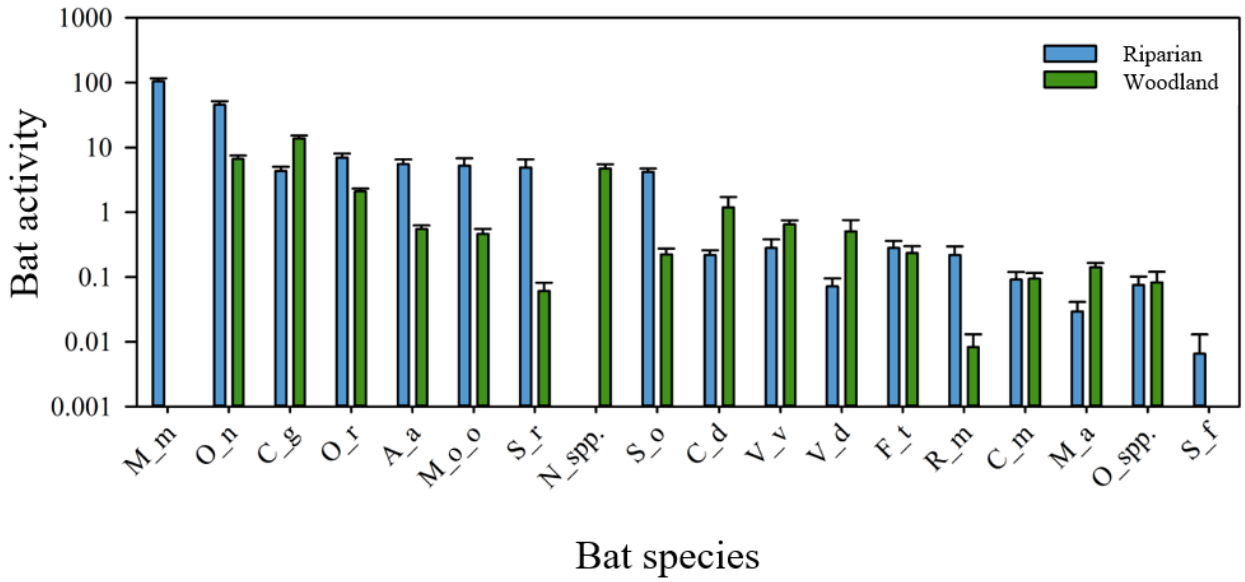

Figure 6. Average bat activity per night (± SD) for individual bat species, among the four sites for the riparian versus woodland habitat types. Species ordered by total activity from both habitat types. Graph scale log10 transformed. Bat species names: M_m = Myotis macropus, O_n = Ozimops norfolkensis, C_g = Chalinolobus gouldii, O_r = Ozimops ridei , A_a = Austronomus australis, M_s_o = Miniopterus orianae oceanensis, S_r = Scoteanax rueppellii , N_spp. = Nyctophilus spp., S_o = Scotorepens orion, C_d = Chalinolobus dwyeri, V_v = Vespadelus vulturnus, V_d = Vespadelus darlingtoni, F_t = Falsistrellus tasmaniensis, R_m = Rhinolophus megaphyllus, C_m = Chalinolobus morio, M_a = Miniopterus australis, O_sp. = Ozimops sp., S_f = Saccolaimus flaviventris.

To test whether the influence of season and air temperature differed among different species of bats, I applied another LME to a subsample of the data that tested for an interaction between species and air temperature. In this model, a subset of the data was used that included only the 6 species with the highest recorded number of calls that also occurred at ≥ 50 % of sites because including species with relatively few record calls adversely affected the performance of the model to accurately distinguish the effect of species. The reduced LME model indicated a significant and strong interaction between the effects of species and air temperature (Table 3). The model's predicted mean partial effects of species suggested that

activity increased more strongly as a function of increasing air temperature for two species: *A. australis* and *C. gouldii*, compared to the other species, and that an effect of air temperature was weakest in the riparian specialist, *M. macropus* (Figure 7).

Table 3. Results of a linear mixed effects model fitted to explain the variation in the main effects on nightly bat activity among different species. Only the 6 species with the highest recorded calls that also occurred at ≥ 50 % of sites were included in the model (*M. macropus*, *O. norfolkensis*, *C. gouldii*, *O. ridei*, *A. australis* and *M. o. oceanensis*). Site was treated as a random effect on the intercept. The initial global model included the following non-significant fixed effects that were removed sequentially to derive the final model: Rainfall, Rainfall^2 and Wind^2.

Fixed effect	**Chisq**	**Df**	**Pr(>Chisq)**
(Intercept)	8.0	1.0	0.005
Air temperature (°C)	27.3	1.0	<0.001
Species	83.6	5.0	<0.001
Habitat type (riparian)	0.3	1.0	0.579
Air temperature^2 (°C)	23.2	1.0	<0.001
Wind (m/s)	4.4	1.0	0.036
Day of year (DOY)	1.7	1.0	0.191
Sin (2π/365 × DOY)	65.8	1.0	<0.001
Cos (2π/365 × DOY)	8.9	1.0	0.003
Air temperature (°C) × Species	40.2	5.0	<0.001
Habitat type (riparian) × DOY	16.2	1.0	<0.001

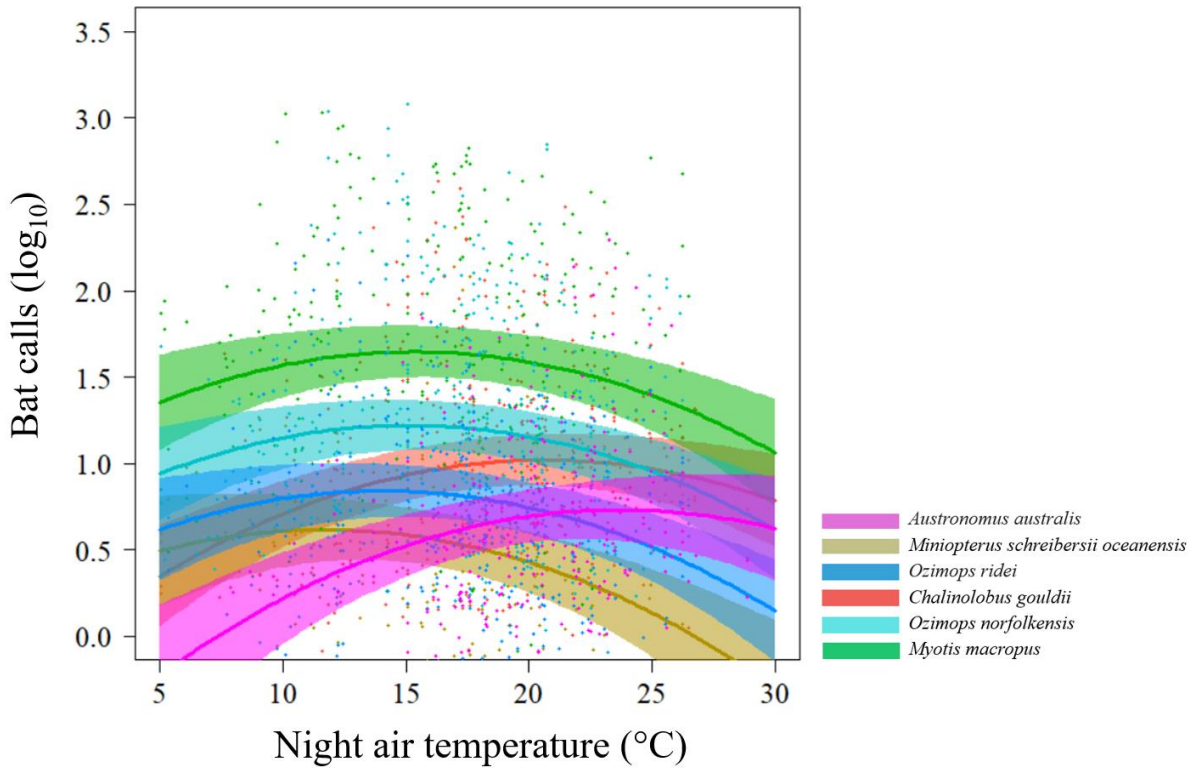

Figure 7. Partial effects plot from linear mixed effects model fitted to explain nightly variation in bat activity (log_{10}) among different species, as a function of night air temperature (°C). The different coloured symbols and lines show the predicted effect of species on the slope of the quadratic effect of air temperature on average nightly bat activity. For all effects see model results shown in Table 3.

Based on the significant interaction of species and air temperature, I explored species-specific effects by fitting separate models to subsets of the data for each species. I tested for effects only using data for the particular species which revealed a significant positive quadratic effect of mean nightly air temperature (°C) on bat activity was apparent only for *O. norfolkensis*, *C. gouldii* and *O. ridei* (Table 4, Figure 8). There was no relationship between temperature and activity for *M. macropus*, *A. australis,* and *M. orianae oceanensis*. Furthermore, wind speed had a negative effect on the activity of *O. norfolkensis* and *O. ridei* but not for the other species.

Table 4. Results of separate linear mixed effects models fitted to subsets of the data for each of the six most commonly recorded bat species. Fixed effects that were significant in any of the models were then retained in all models for each species to allow effect sizes to be more comparable. However, because *Myotis macropus* was only present in riparian sites, habitat type could not be included as a fixed effect in this species' model. Site was treated as a random effect on the intercept. The initial global model included the following non-significant fixed effects that were removed sequentially to derive the final model: Rainfall, Rainfall^2 and Wind^2.

Species	Fixed effect	Coeff	SE	DF	t-value	*p*-value
Myotis macropus	(Intercept)	0.642	0.5	10.1	1.3	0.220
	Air temperature (°C)	0.033	0.0	264.1	0.8	0.399
	Air temperature^2 (°C)	-0.001	0.0	264.1	-1.0	0.305
	Wind	0.052	0.1	264.0	0.6	0.547
	Day of year (DOY)	0.003	0.0	264.3	4.7	<0.001
	Sin (2π/365 × DOY)	0.494	0.1	264.1	5.1	<0.001
	Cos (2π/365 × DOY)	0.108	0.1	264.1	1.2	0.233
Ozimops norfolkensis	(Intercept)	-0.346	0.3	194.1	-1.1	0.295
	Air temperature (°C)	0.105	0.0	450.0	2.9	0.004
	Air temperature^2 (°C)	-0.003	0.0	449.9	-2.9	0.005
	Wind	-0.250	0.1	450.7	-3.4	0.001
	Habitat type (riparian)	0.619	0.2	6.0	3.5	0.013
	Day of year (DOY)	0.000	0.0	453.5	0.0	0.990
	Sin (2π/365 × DOY)	0.592	0.1	449.8	7.3	<0.001
	Cos (2π/365 × DOY)	-0.010	0.1	451.0	-0.1	0.894
Chalinolobus gouldii	(Intercept)	-0.564	0.3	445.4	-1.6	0.103
	Air temperature (°C)	0.152	0.0	462.3	4.0	<0.001
	Air temperature^2 (°C)	-0.004	0.0	462.7	-4.0	<0.001
	Wind	-0.077	0.1	458.1	-1.2	0.226
	Habitat type (riparian)	-0.337	0.1	5.3	-4.1	0.008
	Day of year (DOY)	0.000	0.0	453.2	0.9	0.371
	Sin (2π/365 × DOY)	0.199	0.1	462.6	2.5	0.012
	Cos (2π/365 × DOY)	0.107	0.1	458.0	1.6	0.114
Ozimops ridei	(Intercept)	-0.491	0.4	340.4	-1.3	0.192
	Air temperature (°C)	0.140	0.0	343.5	3.3	<0.001
	Air temperature^2 (°C)	-0.004	0.0	343.0	-3.3	<0.001
	Wind	-0.235	0.1	344.6	-2.7	0.007
	Habitat type (riparian)	0.250	0.1	6.1	2.7	0.036
	Day of year (DOY)	0.000	0.0	346.7	1.0	0.334
	Sin (2π/365 × DOY)	-0.052	0.1	346.9	-0.6	0.567
	Cos (2π/365 × DOY)	-0.081	0.1	344.4	-1.1	0.278
Miniopterus orianae oceanensis	(Intercept)	0.240	0.5	144.0	0.5	0.609
	Air temperature (°C)	0.007	0.1	155.0	0.1	0.888
	Air temperature^2 (°C)	-0.001	0.0	154.8	-0.6	0.520
	Wind	-0.115	0.1	155.4	-1.0	0.305
	Habitat type (riparian)	0.047	0.2	6.2	0.3	0.811
	Day of year (DOY)	0.001	0.0	157.6	1.3	0.187

	Sin (2π/365 × DOY)	0.426	0.1	157.6	3.1	0.002
	Cos (2π/365 × DOY)	0.132	0.1	155.4	1.2	0.229
Austronomus australis	(Intercept)	-0.555	0.6	220.4	-0.9	0.371
	Air temperature (°C)	0.014	0.1	217.8	0.2	0.833
	Air temperature^2 (°C)	0.000	0.0	217.9	-0.2	0.870
	Wind	0.138	0.1	216.5	1.8	0.075
	Habitat type (riparian)	0.534	0.1	6.6	5.2	0.002
	Day of year (DOY)	0.001	0.0	220.9	3.6	<0.001
	Sin (2π/365 × DOY)	0.141	0.1	218.4	1.2	0.247
	Cos (2π/365 × DOY)	0.349	0.1	218.4	3.3	0.001

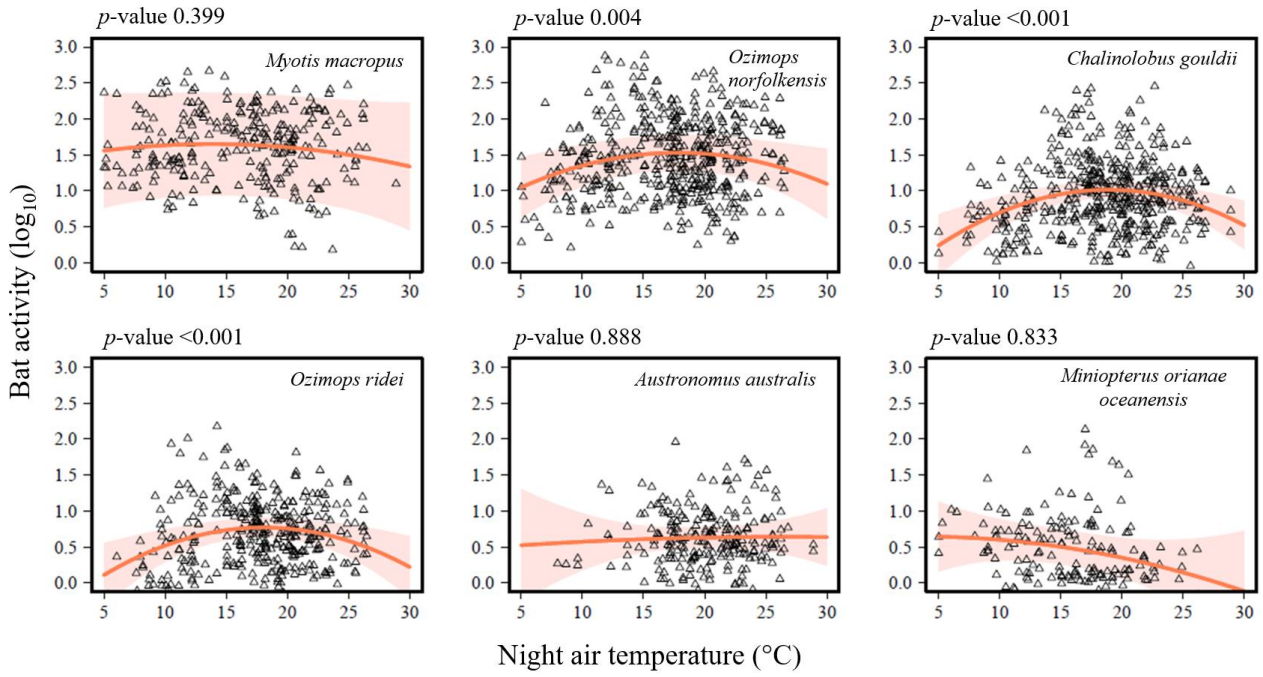

Figure 8. Partial effects plots from separate linear mixed effects models fitted to explain nightly variation in bat activity (log_{10}) as a function of night air temperature (°C), for different species. Partial effects plots show the mean estimated effect of night air temperature on average nightly bat activity for each species. The effect of mean nightly air temperature on bat activity was significant only in models for *O. norfolkensis*, *C. gouldii* and *O. ridei.* For all effects see model results shown in Table 4.

Activity of insects

The nightly mass and count of insects were generally highest in December and decreased throughout the study, with the lowest amounts recorded in July. There was variation in the activity of insect orders between habitat types and among months, with a greater mass and count of Coleoptera in woodland sites and more insects classed as “Other” in riparian sites (Figure 9).

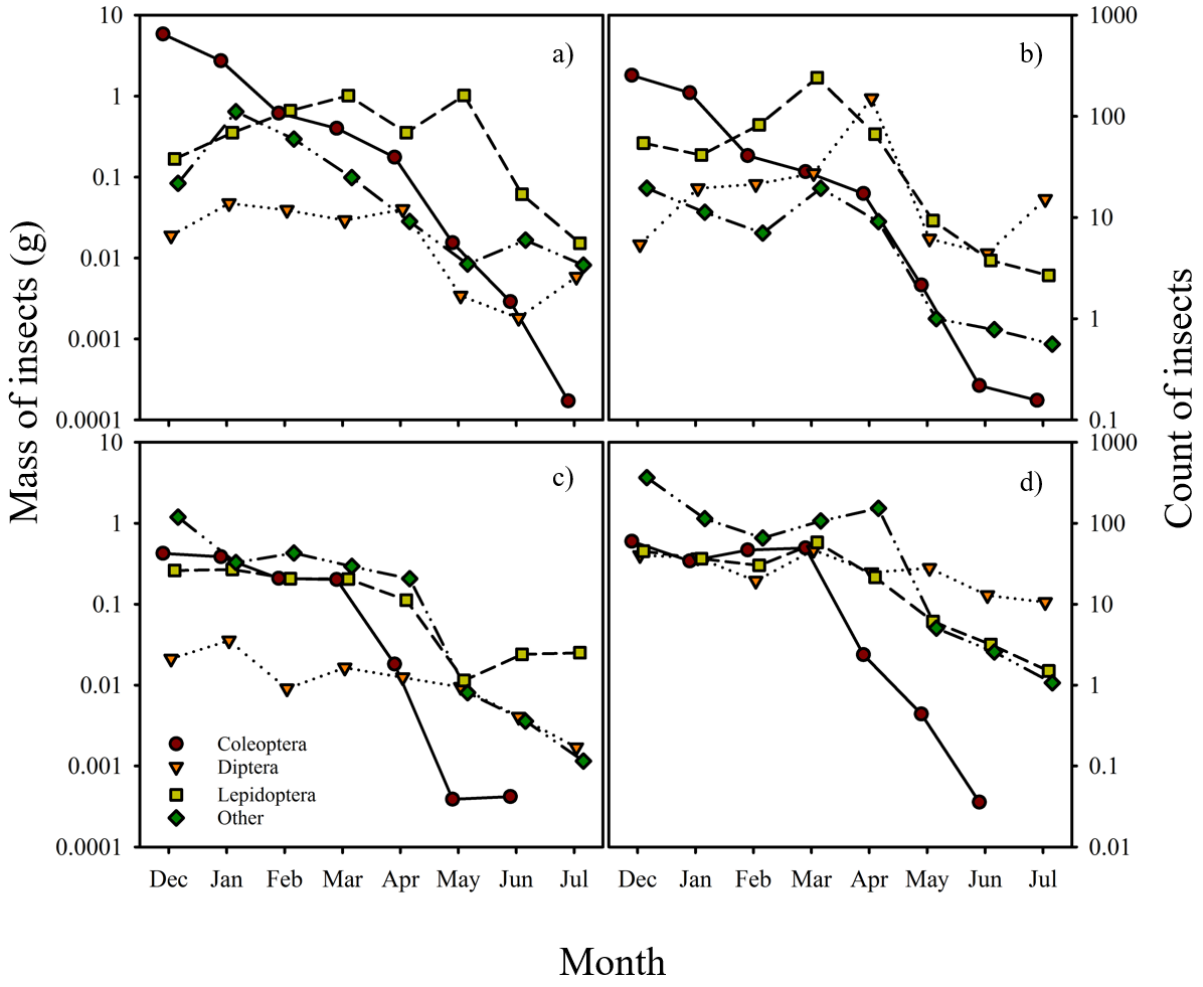

Figure 9. Average mass (g, left panels) or count (right panels) of insects belonging to three identified orders and all other insects caught per night among the four sites for the woodland (a, b) and riparian (c, d) habitats. Graph scale $\log_{10}$ transformed.

The most parsimonious linear mixed effects model fitted to explain nightly variability in insect mass or count included a significantly positive effect of mean nightly air temperature (Table 5, Figure 10). Insect mass and count increased with increasing nightly air temperature. The interaction between habitat type and air temperature indicated a weaker effect of increasing air temperature on insects in the riparian habitat, when measured in mass. However, when insect activity was measured by count, this interaction was not significant. Day of the year affected insects according to a sinusoidal curve, but there was no interaction between habitat type and day of the year. The most parsimonious model also retained a significant but weak negative effect of wind speed.

Table 5. Results of linear mixed effects models fitted to explain variation in insect activity. Site was treated as a random effect on the intercept. Insect activity was measured in mass (g) and count. Size and order were not included in the model as distinguishing classes. Rainfall, Rainfall^2, Wind and Wind^2 were tested and removed from the global model as they were not significant.

Measurement of insect activity	**Fixed effect**	**Coeff**	**SE**	**DF**	**t-value**	***p*-value**
Mass of insects (log_{10}, g)	(Intercept)	-0.419	0.2	80.9	-1.9	0.066
	Air temperature (°C)	0.054	0.0	77.6	3.9	<0.001
	Habitat type (riparian)	0.138	0.1	69.1	0.9	0.346
	Day of year (DOY)	0.001	0.0	83.0	2.1	0.042
	Sin (2π/365 × DOY)	0.010	0.1	80.3	0.1	0.891
	Cos (2π/365 × DOY)	0.166	0.1	77.9	1.6	0.103
	Air temperature (°C) × Habitat type (riparian)	-0.024	0.0	78.1	-2.7	0.008
Count of insects (log_{10})	(Intercept)	0.509	0.3	82.7	1.5	0.134
	Air temperature (°C)	0.096	0.0	78.3	4.7	0.000
	Habitat type (riparian)	0.100	0.2	58.2	0.4	0.664
	Day of year (DOY)	0.002	0.0	82.0	2.4	0.017
	Sin (2π/365 × DOY)	0.418	0.1	79.6	3.9	<0.001
	Cos (2π/365 × DOY)	0.004	0.1	78.4	0.0	0.981
	Air temperature (°C) × Habitat type (riparian)	-0.008	0.0	78.6	-0.6	0.525

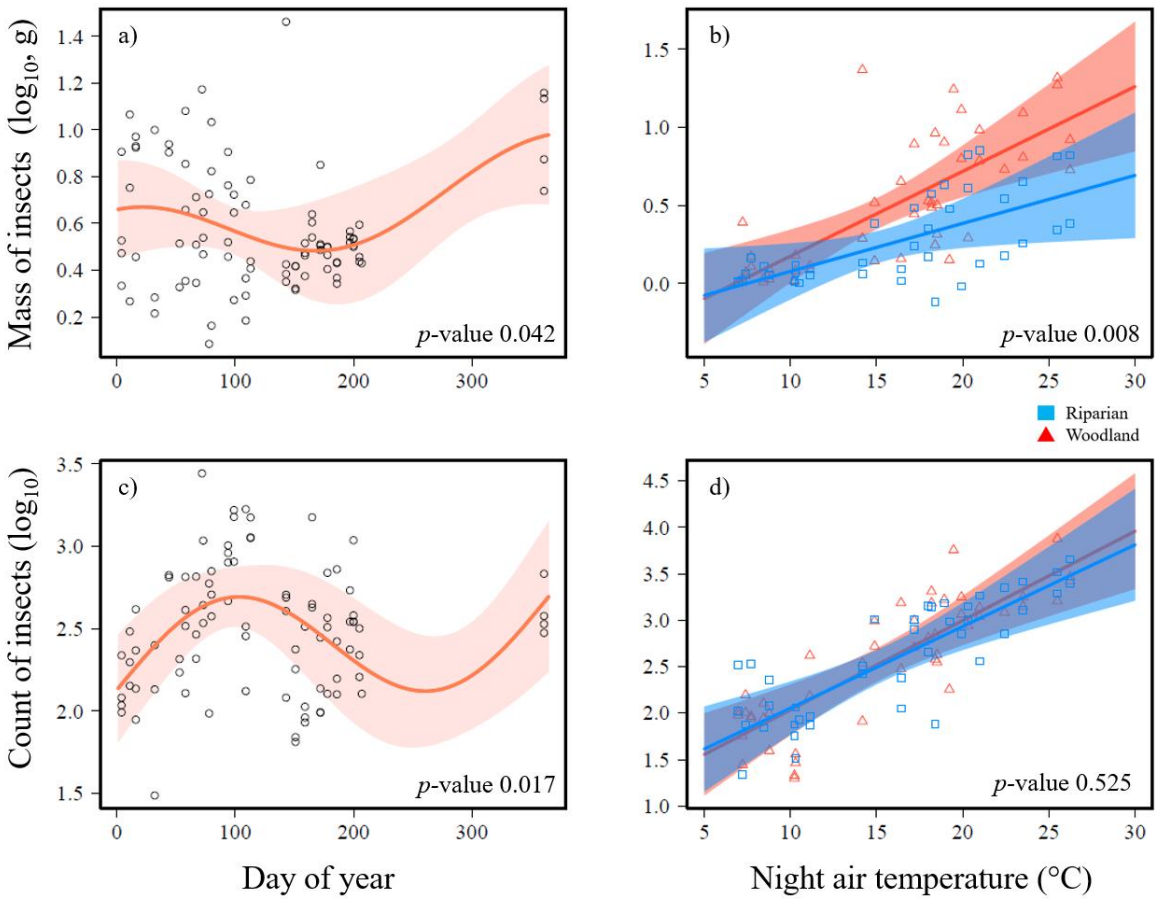

Figure 10. Partial effects plots from separate linear mixed effect models fitted to explain nightly variation in insect mass (log10, g, top panels) and count (log_{10}, bottom panels), as a function of day of year (a, c) and night air temperature (°C)(b, d). Partial effects plots show the mean estimated effect of each explanatory variable that is statistically independent of other fixed effects included in the model. There was no significant difference between habitat types for (c). For all effects see model results shown in Table 5.

Given the variation in insect mass (g) among orders seen in the raw data (Figure 9), I explored the interaction between air temperature and habitat type with insect order and size class. The relationship between count and mass (g) varied among insect classes. For example, Diptera are typically very light so by number Diptera appeared more abundant than they did by mass. Therefore, the most parsimonious linear mixed effects model fitted to explain nightly variability in insects was run using the mass and number for both insect order and insect size (Table 6). All four models included a significant positive effect of mean nightly air temperature. The reduced LME model indicated a significant and strong interaction between the effects of order/size and air temperature. The model's predicted mean partial effects of order and size suggested that activity increased more strongly as a function of increasing air temperature for Coleoptera and that the effect of air temperature was weakest for Diptera and Lepidoptera (Figure 11). Insects in size class 2 (5 to10 mm) had the strongest relationship

with mean nightly air temperature. The model also indicated a significant and strong interaction between the effect of order/size and habitat type. The predicted mean partials effects indicated there were more Coleoptera, Lepidoptera and size 3 and 4 insects in woodland than riparian habitats (10 to 15 mm, > 15 mm) (Figure 12).

Table 6. Results of separate linear mixed effects models fitted to explain the nightly variation in insect activity. The first model relates the total nightly mass of insects in each order (Coleoptera, Lepidoptera, Diptera and other) to mean air temperature. The second model relates the total nightly number of insects in each order to temperature. The third model tests the relationship between the total nightly mass of insects according to size class (<5 mm, 5 to 10 mm, 10 to 15 mm, >15 mm) to mean nightly air temperature. Last, the total nightly number of insects in each size class. Fixed effects that were significant for some species remained in all models. Site was treated as a random effect on the intercept. The reference for order was Coleoptera and the reference for size was class 1 (>5 mm). Rainfall, Rainfall^2, Air temperature^2, Wind and Wind^2 were tested and removed from the global model because they were not significant.

Model variables	**Fixed effects**	**Chisq**	**Df**	**Pr(>Chisq)**
Insect order (mass of insects, log_{10}, g)	(Intercept)	58.8	1	<0.001
	Air temperature (°C)	18.1	1	<0.001
	Order	48.5	3	<0.001
	Habitat type (riparian)	13.9	1	<0.001
	Air temperature^2 (°C)	5.4	1	0.020
	Rainfall	3.0	1	0.081
	Rainfall^2	2.4	1	0.119
	Day of year (DOY)	0.0	1	0.976
	Sin (2π/365 × DOY)	0.0	1	0.886
	Cos (2π/365 × DOY)	3.6	1	0.056
	Air temperature (°C) × Order	57.2	3	<0.001
	Order × Habitat type (riparian)	15.5	3	0.001
Insect order (count of insects, log_{10})	(Intercept)	13.0	1	<0.001
	Air temperature (°C)	13.8	1	<0.001
	Order	87.2	3	<0.001
	Habitat type (riparian)	9.9	1	0.002
	Air temperature^2 (°C)	3.7	1	0.055
	Rainfall	6.9	1	0.009
	Rainfall^2	5.9	1	0.015
	Day of year (DOY)	2.1	1	0.151
	Sin (2π/365 × DOY)	2.5	1	0.112
	Cos (2π/365 × DOY)	3.4	1	0.066
	Air temperature (°C) × Order	77.7	3	<0.001
	Order × Habitat type (riparian)	64.7	3	<0.001
Insect size (mass of insects, log_{10}, g)	(Intercept)	28.4	1	<0.001
	Air temperature (°C)	2.0	1	0.158
	Size	43.3	3	<0.001
	Habitat type (riparian)	10.0	1	0.002
	Air temperature^2 (°C)	0.1	1	0.794
	Rainfall	0.0	1	0.853

	Rainfall^2	0.0	1	0.929
	Day of year (DOY)	5.2	1	0.022
	Sin (2π/365 × DOY)	4.6	1	0.031
	Cos (2π/365 × DOY)	6.4	1	0.011
	Air temperature (°C) × size	31.1	3	<0.001
	Size × Habitat type (riparian)	17.6	3	0.001
Insect size (count of insects, log_{10})	(Intercept)	0.2	1	0.689
	Air temperature (°C)	4.5	1	0.034
	Size	46.0	3	<0.001
	Habitat type (riparian)	2.8	1	0.096
	Air temperature^2 (°C)	1.1	1	0.295
	Rainfall	5.2	1	0.023
	Rainfall^2	4.5	1	0.033
	Day of year (DOY)	3.5	1	0.061
	Sin (2π/365 × DOY)	5.9	1	0.016
	Cos (2π/365 × DOY)	5.1	1	0.024
	Air temperature (°C) × Size	59.4	3	<0.001
	Size × Habitat type (riparian)	16.2	3	0.001

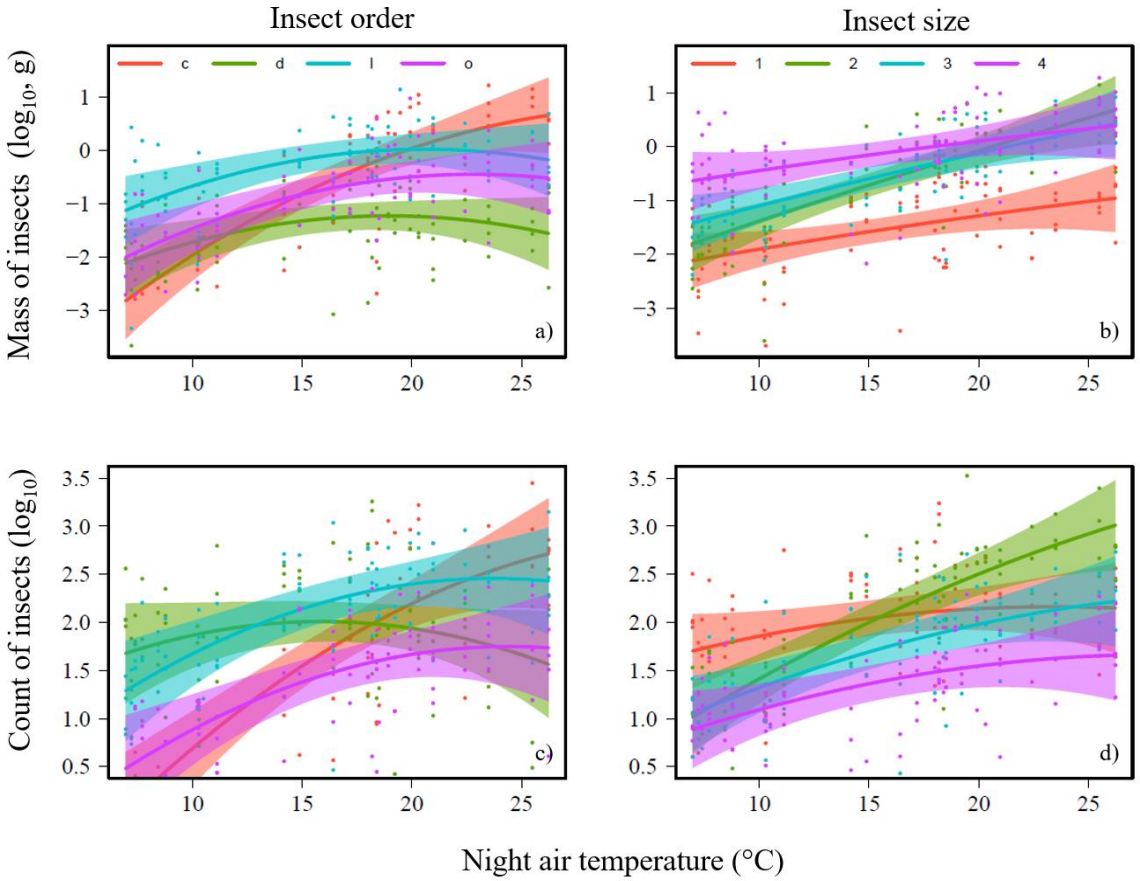

Figure 11. Partial effects plot from separate linear mixed effects models fitted to explain nightly variation in insect mass (log_{10}, g, top panels) and count (log_{10}, bottom panels) among different orders and size classes, as a function of night air temperature (°C). The different coloured symbols and lines show the predicted effect of insect order (a, c) and insect size (b,

d) on the slope of the quadratic effect of air temperature. For all effects see model results shown in Table 6.

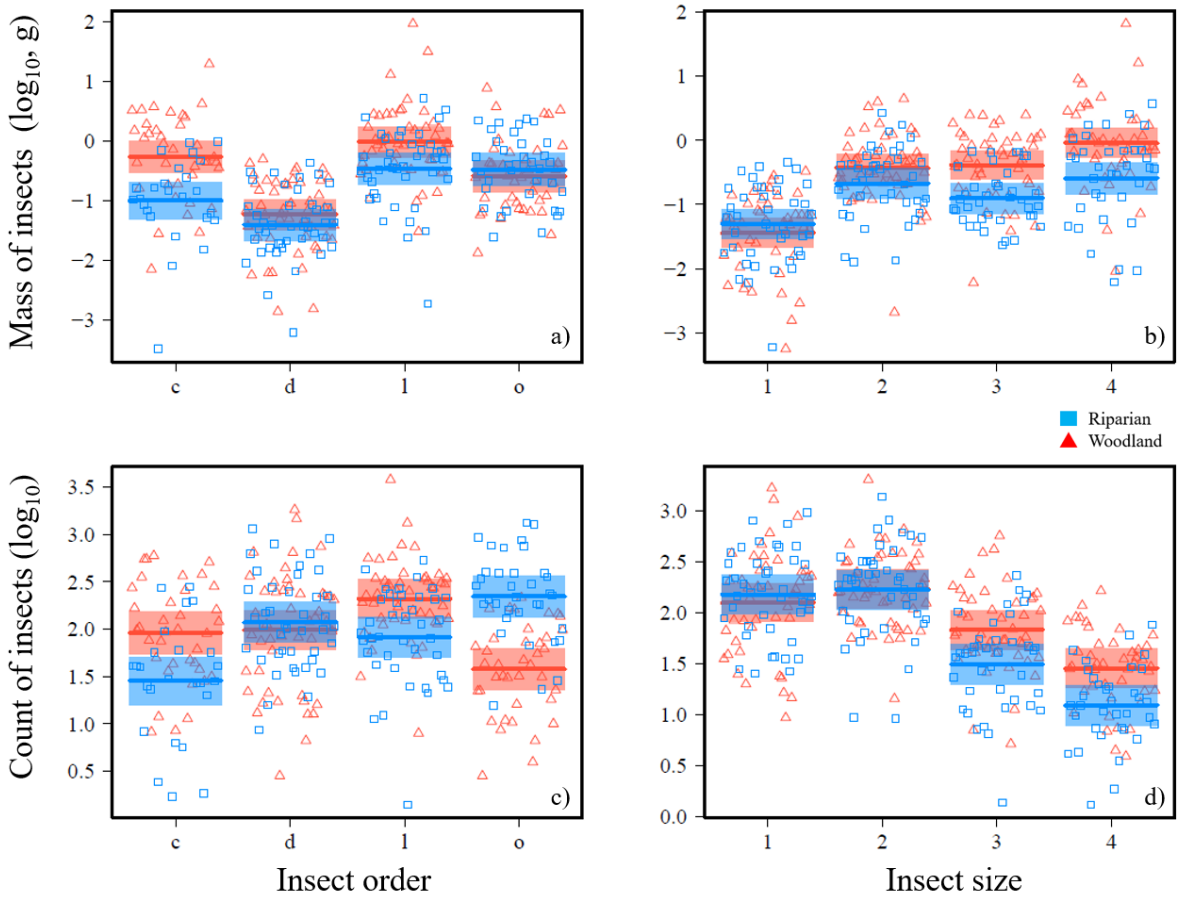

Figure 12. Partial effects plot from separate linear mixed effects models fitted to explain nightly variation in insect mass (log_{10}, g, top panels) and count (log_{10}, bottom panels), as a function of habitat type. The different coloured symbols and lines show the predicted effect of habitat type on insect order (a, c) and insect size (b, d). For all effects see model results shown in Table 6.

Correlating bat and insect activity.

The most parsimonious linear model fitting bat activity as a function of insect abundance showed there was a significant, positive association between bat activity and insects, when insect activity was measured by both mean nightly mass (mg) and by mean nightly count (Table 7, Figure 13). Air temperature was included as a significant interaction term in the models and the partial effects plot showed that a stronger positive relationship was found at lower temperatures. The linear model compared mean nightly bat activity in woodland and riparian sites to the average mass and count of insects collected from woodland

and riparian sites on the same date. Order and size classifications were removed for this analysis as early models revealed order and size were not significant.

Table 7. Results of linear models correlating bat activity (log_{10}) and insect activity (as insect count (log_{10}) and insect mass (log_{10}, mg). Bat activity is the mean nightly bat calls recorded in riparian and woodland habitats, compared to the mean insect mass and count from riparian and woodland sites, on the same date. Day of year (DOY), Rainfall, Rainfall^2, Air temperature^ 2, Wind and Wind^2 were tested and removed from the global model because they were not significant.

Measurement of insect activity	Fixed effect	Coeff	SE	t-value	*p*-value
Bat activity (log_{10}) ~ Mass of insects (log_{10}, mg)	(Intercept)	-1.647	0.4	-4.0	<0.001
	Mass (log_{10}, mg)	0.731	0.2	2.9	0.005
	Air temperature (°C)	0.111	0.0	2.9	0.005
	Habitat type (riparian)	0.538	0.1	3.7	0.001
	Mass (log_{10},g) × Air temperature (°C)	-0.042	0.0	-2.6	0.013
Bat activity (log_{10}) ~ Count of insects (log_{10})	(Intercept)	-1.747	0.4	-4.6	<0.001
	Number (log_{10}):	1.390	0.3	4.0	<0.001
	Air temperature (°C)	0.118	0.0	3.4	0.001
	Habitat type (riparian)	0.513	0.1	4.2	<0.001
	Count (log_{10}) × Air temperature (°C)	-0.077	0.0	-3.4	0.001

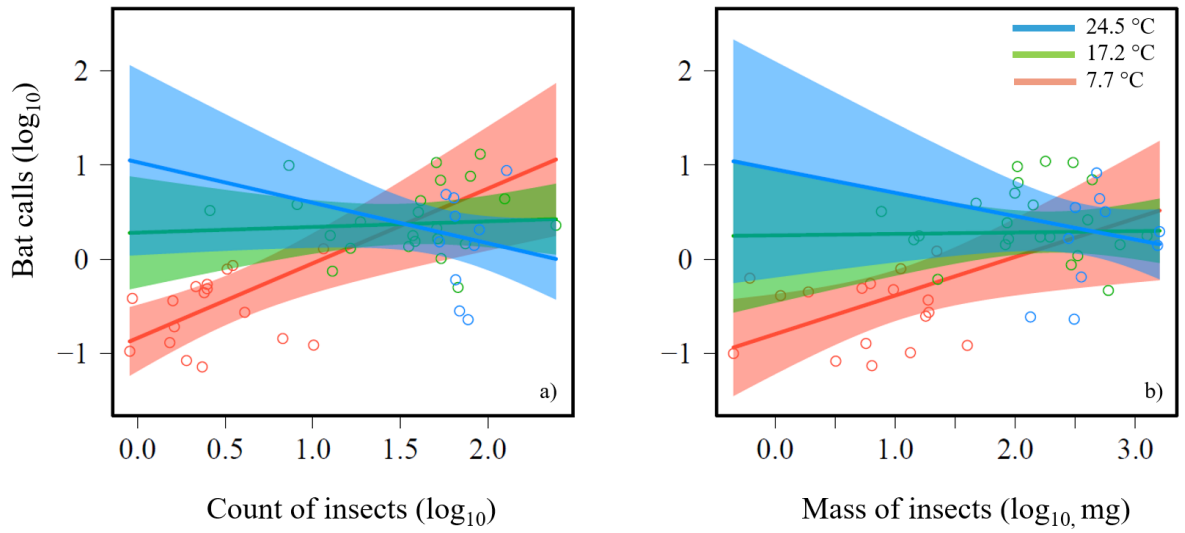

Figure 13. Partial effects plot from separate linear models fitted to explain bat activity as function of a) count (log_{10}) and b) insect mass (log_{10}, mg), with a significant interaction with air temperature (°C). The different coloured symbols and lines show the predicted effect of temperature on the relationship between bat activity and insects (mass/count). For all effects see model results shown in Table 7.

2.4 Discussion

This study assessed how environmental factors, such as air temperature, season and habitat, affect the activity of bats and the abundance of their insect prey. Both bat activity and insect abundance varied among seasons, with both variables declining from mid-autumn through to winter. After accounting for this seasonal effect, bat and insect activity were positively associated with mean nightly air temperature (°C); however, bat activity followed a hump-shaped pattern and tended to decrease on the warmest nights when air temperature exceeded 22 °C. Bat activity was positively associated with insect activity only for relatively cool nights, suggesting that on milder nights, bats and insects respond independently to environmental conditions. The relationship between activity and temperature differed between habitat types, among bat species and among different orders and sizes of insects. Activity of the riparian specialist, *M. macropus*, was not strongly related to nightly air temperature. This study is valuable because it sampled both intensively and long-term at replicated sites within two distinct habitat types, which allowed the distinction of patterns in bat and insect activity both within habitats, among days, seasons and among different habitats. Understanding what promotes the timing of bat activity is an important aspect of ecology because it helps us manage ecosystems to sustain bat populations and design surveys that accurately represent population numbers.

Relationships between bat and insect activity with temperature

Activity of bats and insects generally increased with increasing nightly air temperature. A positive relationship between bat activity and temperature is consistent with other findings (O'Donnell 2000; Erickson & West 2002; Scanlon & Petit 2008; Bender & Hartman 2015). The response of bat activity to temperature was curvilinear with reduced activity below 15 °C and the positive effect of increasing air temperature diminishing above 15 °C. Similarly, a study in tropical Queensland found that 99.7 % of recorded bat calls were made above 15 °C (Richards 1989). In other studies, bat activity was greatly reduced at temperature below 10 °C (Agosta *et al*. 2005; Wolbert, Zellner, and Whidden 2014). In the present study, when air temperature increased beyond 22 °C there was a slight decrease in bat activity, especially in maximum calls detected. An upper temperature threshold for bat activity has been found in other studies, however, the temperature at which bat activity decreased varied between studies: 15 to 30°C (Wolcott & Vulinec 2012), 21°C (Brooks 2009), 19°C (Wolbert, Zellner and Whidden 2014). The variation might be attributed to the local adaptation of bats to temperature where the studies were performed. Insect

activity had a significant positive relationship with temperature, that unlike bat activity was linear. A positive correlation between insect activity and air temperature is consistent with previous studies (Ward & Stanford 1982; Meyer, Senulis & Reinartz 2016; Brooks, Loeb & Gerard 2017). Slightly lower bat activity at high temperatures may be because bats did not need to spend as much time foraging on warm nights as high insect activity made hunting relatively easy and they were able to consume an ample amount of food quicker than on nights with less insect activity. Generally, both bats and their insect prey were strongly impacted by temperature in my results.

Seasonal variation in bat and insect activity

Day of year, or season, had a significant effect on bat activity, independent of the modelled effect of nightly variation in temperature. For mammals, season shapes food availability, roosting conditions, breeding times and much more, as well as obvious changes in air temperature (Morrison 2006). So, it is was expected that bat activity would be significantly associated with time of year. In this study, bat activity increased from December to March before decreasing between April and July. Similarly, total bat activity was shown to be greatest in autumn elsewhere in Sydney, though surprisingly activity in winter was not significantly different to spring or summer (Gonsalves & Law 2018). Autumnal peaks in activity could be driven by a need to increase energy stores for winter or breeding (Kunz, Wrazen & Burnett 1998), prior to a decrease in winter bat activity reflecting the extended torpor use and hibernation of many species (Geiser& Brigham 2000; Turbill 2008; Stawski & Currie 2016). Season is a significant driver of bat activity.

Insects had a strong positive association with temperature and although there is an apparent decrease in insects heading into winter, in a model including multiple effects it seems much of this trend is explained by the lower air temperature in winter, with a weaker effect of season alone. When insect activity was subcategorised into order and size classes the effect of season was not significant for all models except when the size of insects was measured by mass. This could mean there was a greater effect of season on large insects, as these are better represented when activity was measured by mass. Season has been seen to significantly affect insect activity (Wolda 1978; Wickramasinghe *et al*. 2004; Pawson, Marcot & Woodberry 2017) however, daily weather variables, rather than season, has been recorded as having greatest influence on insect activity (Grüebler, Morand & Naef-Daenzer 2008). Insect and bat activity do not seem to share the same strength of response to season.

The influence of habitat type on bat activity and insect activity

Habitat type was shown to influence nightly variation in bat activity in three ways. Firstly, there was significantly higher bat activity in riparian sites. The high abundance of *M .macropus* that are present and restricted to waterways might have boosted the activity recorded in riparian sites, generally however, bat activity has been positively associated with nearby water sources (Russo & Jones 2003; Kahnonitch, Lubin & Korine 2018). Waterways are generally more open and unobstructed than woodlands, providing flight paths and foraging areas that are less energetically expensive and difficult for bats to manoeuvre (Law & Chidel 2002; Rainho, Augusto & Palmeirim 2010; Almenar *et al*. 2013; Brooks, Loeb & Gerard 2017). Also, bat activity has been seen to increase with relative humidity (Wolcott & Vulinec 2012), so the more humid conditions in riparian zones may be bolstering bat activity. Increased humidity and resources associated with riparians zones, and thus increased insect activity, might be driving the high rates of bat activity in riparian habitats (Grindal, Morissette & Brigham 1999; Law & Chidel 2002).

Secondly, as predicted, riparian bat activity showed a weaker relationship to air temperature than woodland bat activity. Gonsalves & Law (2017) found no relationship with temperature for bat activity over water, however, as the aim of their study was to survey the presence of species, they intentionally avoided unfavourable conditions (ie., wind, rain or especially cold nights), didn't sample over winter or sample repeatedly in sites to ascertain within site variability. Furthermore, there was no woodland habitat sites sampled as a comparison. My experimental design focused on determining thermal relationships in a rigorous manner to confirm that bat activity over waterways in NSW are less affected by temperature than woodland habitats. Bats foraging in riparian habitats might benefit from the higher thermal inertia of water, creating a stable environment for insects, thus more consistent prey availability (Portner 2002; Frayne 2013). When measured by mass insect activity has a significantly weaker relationship with air temperature in riparian habitats than terrestrial, however, there was less total mass of insect in riparian habitats (likely due to a greater number of small insects found in riparian sites). In this study, bat activity was measured as the total number of recorded calls per night, which may include commuting, drinking or foraging activity. Hydration levels can cause winter arousals by bats (Ben-Hamo *et al*. 2012; Ben-Hamo *et al*. 2013), so bat activity detected over water might be associated with drinking, and thus take place irrespective of temperature and is unlikely to be recorded in woodland sites. Further work is needed to disentangle the influence of drinking activity on

total bat activity, however, my results support the hypothesis that riparian zones are decreasing thermal effects on bats and insect prey.

Woodland bat activity had a stronger decline over winter compared to riparian bat activity. However, season was not significantly different between habitat types for insects, likely due to the weak effect of season found for overall activity. This contrasts other studies as the spring emergence of aquatic insects in Japan was considered one of the most important drivers of bat foraging in the area. And seasonal abundance of aquatic insects peaked in spring, compared to summer for woodland insects (Fukui *et al*. 2006). The decreased effect of temperature on insect activity recorded in my study could have influenced the winter availability of prey in riparian zones. Also *M. macropus* were highly abundant in riparian sampling sites so overall riparian bat activity could be influenced by the species-specific traits of *M. macropus*. Further investigation is needed to fully understand the influence of habitat type for insectivorous bats and their prey.

There was greater activity of small insects and Diptera in riparian habitats compared to woodland habitats, which had a greater proportion of Coleoptera, Lepidoptera, and larger insects. This is consistent with findings from other studies (Thomas 1988; Rydell, Entwistle & Racey 1996). When comparing bat activity and insect biomass between saltmarsh and coastal swamp forest, small insects (< 2mm) constituted most of the insect biomass found in saltmarsh, whereas Lepidoptera and Coleoptera formed the majority of coastal swamp forest insect biomass (Gonsalves *et al.* 2013b). The high activity of small prey in riparian zones is more beneficial for small bat species that are capable of the detection and gaining profitability from small prey. (Gonsalves *et al.* 2013b). Possibly higher bat activity in riparian zones can be attributed to longer foraging times by bats feeding on a many small prey, compared to bats feeding on a lesser number of larger prey in woodland habitats.

Species-specific responses of bat activity to variation in nightly air temperature

As predicted, Australia's only fishing bat, *M. macropus* did not have a significant relationship with temperature. There could be a number of reasons for this, but it is likely to reflect the decreased influence of temperature on insect activity in riparian habitats. Also, *M. macropus* might have shown no relationship to temperature because they have constant access to water, so there is little restriction on evaporative cooling, thus excess heat might not negatively impact their activity. Season had a significant effect on the activity of *M. macropus*, with a decrease in activity over winter like other bat species. The effect of season

on insects was not consistent among insect models, so seasonal changes in the activity of *M. macropus* might be more related to seasonal changes in energy demands associated with reproduction and thermoregulation during rest. *Myotis macropus* could also be relying on piscivory to supplement their diet when inset abundance is low. However, this idea is untested and further research into the seasonal diet of *M. macropus*, particularly using molecular methods which may be more reliable for detecting fish remnants, could be beneficial. Furthermore, my study did not test the activity of water bourn insects or small fish, so further study on the seasonal abundance of these prey types would be beneficial for understanding prey availability for fishing bats. The hypothesis that riparian habitats have a decreased thermal affect can be supported from looking at total bat and insect activity in riparian habitats and at the species-specific response of the riparian specialist bat *M. macropus.*

As predicted, there was a positive relationship between air temperature and nightly activity for non-riparian specialist species *Chalinolobus gouldii*, *Ozimops norfolkensis* and *Ozimops. ridei*. The strength of these relationships differed, with *C. gouldii* showing the strongest relationship, followed by *O. ridei* and lastly, *O. norfolkensis*. Activity was not significantly influenced by air temperature also for *Miniopterus orianae oceanensis* and *Austronomus australis,* which was not predicted. As roost microclimates are highly influential on bat behaviour (Campbell, Coulson & Lumsden 2010) it is possible *M. o. oceanensis* showed less influence with temperature because they roost in well insulated cavities or caves (Codd, Sanderson & Branford 2003). *Austronomus australis* has shown preference for foraging over wetlands (Straka *et al*. 2016) and may be less influenced by temperature fluctuations due to the thermal benefits of foraging in riparian ecosystems. Furthermore, *A. australis* is a large bat (38 g) so they are capable of foraging for larger insect prey, like endothermic moths which might be active at a greater range of temperature (Churchill 2006). Also due to their greater body mass *A. australis* may be less impacted by thermal energetic constraints than smaller bats recorded in the study. Alternatively, the migration patterns of *A. australis* (Churchill 2009), and of *M. o. oceanensis* (Dwyer 1963; Gonsalves & Law 2017b), as well as being the two lowest recorded bats included in the subset of abundant species, may confound associations between activity and temperature.

Sensitivity to temperature amongst insect types

Insects responded differently to temperature depending on their size and order. There was a curvilinear response by some insect orders to temperature that was not visible when looking at insect activity as a whole. Coleoptera had the strongest relationship with temperature, whereas Lepidoptera and Diptera were less sensitive to changing temperature. The stronger relationship between overall insect activity and temperature in woodland compared to riparian sites could be linked to the greater abundance of Coleoptera in woodland sites. A weaker relationship with temperature for insect orders Lepidoptera and Diptera might be because Lepidoptera have shown behavioural and physiological methods of metabolic heat generation, i.e. endothermic thermoregulation (Heinrich 1974; Rawlins 1980; Hegna *et al*. 2013), which enables them to be active at cool air temperatures. Whereas, Diptera were most active in riparian sites and potentially benefited from the thermal inertia of water and increased humidity, which could have been beneficial particularly in dealing with the hottest nightly conditions.

Insects that were 5 to 10mm in length (size class two) were most strongly associated with air temperature compared to other size classes in my study. Generally smaller insects are capable of flight with lower internal temperatures, compared to larger insects (Heinrich 1974; Bennett 1993). However, thermoregulatory capability is positively associated with increasing body mass (Bartholomew & Heinrich 1973). Therefore, although smaller insects are capable of flight with colder internal temperature, larger insects are more likely to be able to increase their internal temperature by shivering prior to activity. These opposing mechanisms could explain why the next largest size class of insects (10 to 15 mm) exhibited the greatest sensitivity to temperature in my study as they did not benefit from either adaptation.

Effect of wind and rainfall on bat and insect activity

Rainfall had no effect on bat activity in any models. Although no relationship between bat activity and rainfall has also been reported by some other studies (Scanlon & Petit 2008), there is evidence to suggest that rainfall can lower bat activity (Erickson & West 2002). In my study the effect of rainfall on insect activity varied depending on the measure of insect activity that was used (i.e., counts or mass). When measuring insect activity as counts, rainfall was a significant positive variable. However, when insect activity was measured by mass, there was no relationship with rainfall. Perhaps the discrepancy between count and mass when considering small compared to large insects is responsible for this anomaly.

Colonies of smaller insects were more damaged than larger insects in a simulated rainfall experiment (Zimmermann & Moran 1991). This suggests that we can see the significance of rain on insect populations when using count rather than mass, as counting insects is more representative of these smaller insects, which are more effected by rain. In my study, there was a weak negative effect of wind on total bat activity, which is congruent with other studies (Wolcott & Vulinec 2012). Wind was not significant in any insect models and was thus removed to increase the parsimony of the models. The lack of significance contradicts evidence that wind typically has a negative effect on insect activity (Grüebler, Morand & Naef-Daenzer 2008). Rainfall and high wind speeds were not common throughout the study period so there may not have been enough data collected under these conditions to establish a strong effect on insect or bat activity.

Relationships between insect and bat activity

Bat activity was positively associated with increasing insect activity, when mean nightly air temperature was included as an interaction term in the model. There was a stronger positive relationship between bat and insect activity when air temperature was lower. In previous studies, the relationship between bat activity and insect activity has had varied outcomes. There has been significant positive (Hayes 1997) and negative (Wolbert, Zellner & Whidden 2014) associations, as well as, no relationship (Grindal, Morissette & Brigham 1999; Wickramasinghe *et al*. 2004). Possibly climate and location of studies influence bat response to insect activity. Although season was insignificant in the linear models fitting bat activity as a function of insect activity in this study, it might be responsible for the discrepancies among other research as seasonal changes in energetic demands might influence the reliance of bats on insect prey.

Conclusion

Understanding the complex interactions that insectivorous bats have with environmental conditions is an ongoing area of small mammal ecology. Although my study was conducted in only one location, it is important because intensive and continuous sampling at replicate sites, over a long period of time, is relatively rare in bat ecology. Thus, my data set allows both a comparison among days within a season and among different seasons, and in addition a comparison of these temporal effects on bat and insect activity between two habitat types. My study demonstrates how influential habitat type is on thermal relationships and predator prey dynamics. Moreover, it has highlighted the benefits and thermal tolerance provided by

riparian systems for both insectivorous bats and insect prey, two key organisms in ecosystem function and agricultural success. With the increasing intensity of drought predicted to be caused by climate change and perhaps ongoing degradation of riparian systems by excessive water extraction for agriculture and mining across Australia, it is essential to conserve waterways because they minimise the impact of thermal conditions on bats and provide essential resources for predator and prey alike.

Chapter 3: Torpor and activity during winter by Australian fishing bats, *Myotis macropus*

3.1 Introduction

Energetics is important to understanding animal ecology because it dictates foraging behaviour, breeding patterns and species survival in the face of changing environmental conditions. Small endotherms have very high mass-specific metabolic rates and therefore balancing energy budgets can be particularly challenging (Brown *et al*. 2004). Energy saving mechanisms, especially torpor – the controlled reduction in body temperature and resting metabolism, are an integral aspect of physiological ecology of many small mammal (Geiser 2004). The thermoregulatory behaviour of small mammals is influenced by important environmental factors, like season, temperature and habitat type, and understanding these relationships is an ongoing area of study. Torpor is used extensively by small insectivorous bats (Speakman & Thomas 2003), because it helps resolve the dual challenge posed by low air temperatures; high thermoregulatory energy costs but low availability of flying insect food. Knowledge on torpor use by bats is increasing, however there is still little known about torpor use outside of cold temperate habitats. And the importance of torpor to bats that specialise in foraging over riparian habitats, which provides different thermal conditions and more consistent prey availability than woodland habitats.

The resting energy expenditure of small mammals is high because mass-specific metabolic rate increases exponentially with a decrease in body mass (Schmidt-Nielsen & Knut 1984; Suarez 1992). In addition, because the surface area to volume ratio also increases with a reduction in body mass, small mammals also have high mass-specific rates of heat loss in the cold (Chew, Lindberg & Hayden 1967; Suarez 1992; Withers 1992). Therefore, homeothermic thermoregulation can be energetically expensive even at air temperature close to the lower limit of their thermal neutral zone (Geiser 2008). The high energetics costs associated with small endothermic metabolism means they require a relatively high rate of food intake to sustain these demands. Generally, food is not always available, often reduced seasonally (e.g during winter) and for some food types, like insects and nectar, on a daily basis depending on short term changes in environmental conditions. Lower food availability has an increased impact on smaller animals because they are incapable of carrying substantial fat reserves (Withers 1992), so even day-to-day variability in temperature and food availability can put a strain on energetic reserves.

A range of small mammals and birds can use torpor to reduce their energy requirements during periods of low food availability (Ruf & Geiser 2015). Torpor is a temporary, controlled reduction in metabolism and body temperature that is associated with reducing the thermoregulatory set-point by endothermic animals (Wang & Wolowyk 1988; Barclay, Lausen & Hollis 2001). Torpor provides a mechanism for the conservation of energy that would be required to maintain a higher thermoregulatory threshold when ambient conditions are not favourable. Torpor bouts can be used for short periods of time, often coinciding with the normal resting phase of the day or, can last multiple days and up to several weeks at a time when used by some species during a period of seasonal dormancy called hibernation. The depth of reduction in body temperature and metabolism is greater in species that are capable of using longer torpor bouts (Ruf & Geiser 2014). The use of torpor to reduce daily energy requirements can have physiological and ecological functions that extend beyond a response to low food availability, as it can also serve to reduce water requirements and decrease predation rates (Geiser & Brigham 2012; Turbill & Stojanovski 2018) Furthermore, torpor can be used to slow pregnancy development until conditions can support the high energy demands of lactation (Geiser & Brigham 2012). For many small mammals, torpor is an essential physiological ability and strongly influences seasonal and daily activity patterns.

Insectivorous bats face a dual problem with decreasing air temperature in balancing their energy budget. Firstly, bats are mostly very small, with 41 % of species weighing less than 10 g (Smith *et al*. 2003), and therefore they have high resting metabolic rates, and high additional costs for thermoregulation. Secondly, the activity of their insect food is generally positively associated with temperature (Mellanby 1939; Raimondo 2004) and also affected by season (Wolda 1978; Wickramasinghe *et al*. 2004; Pawson, Marcot & Woodberry 2017). Therefore, in cold weather insectivorous bats must manage increasing metabolic demands with declining prey availability. Torpor greatly reduces energy costs and bats using torpor, rather than maintaining homeothermy, can reduce energy consumption by 50 to 99 % (Geiser, Fritz & Stawski 2011). At 26 °C the metabolic rate of torpid bats was 84 % less than the metabolic rate of bats that were resting at a normothermic temperature in the same conditions (Turbill 2009). Hence, small insectivorous bats demonstrate a strong proclivity and reliance on torpor to conserve energy and torpor has been documented in a wide range of bats living both in cold-climates and warm tropical regions (Geiser, Fritz & Stawski 2011).

Bats use torpor substantially during unproductive seasons (i.e, winter) to reduce thermoregulatory costs when resting and during active phases in response to short-term changes in environmental conditions making foraging unproductive (Speakman & Thomas 2003). In cold climates bats will use torpor regularly throughout winter, usually entering torpor every day and sometimes remaining torpid for multiple days in especially cold conditions (Wermundsen & Siivonen 2010; Stawski & Currie 2016). Energy conservation from torpor increases with the depth and duration of torpor and heterothermic animals can lower their body temperature further when ambient temperature is lower. Thus, low colder conditions can increase energy savings (Heldmaier, Ortmann & Elvert 2004). In one study on hibernating bats, the average loss in body mass over 70 days was only 13 to 14 % (Liu & Karasov 2011). Frequent and prolonger torpor bouts have also been seen during winter in subtropical climates (Stawski, Turbill & Geiser 2009). The thermal neutral zone for small bats is around 30 °C and costs for thermoregulation increase rapidly once ambient temperature falls below this point (Geiser & Brigham 2000). Therefore, the energetic budgeting of small bats is narrow even in mild environmental conditions.

While some more recent studies have found torpor is also used in milder climates it remains uncertain however how and to what extent bats use torpor in mild subtropical climates where winter seasonal changes are less severe. Furthermore, it is unknown how bats use torpor during winter in riparian habitats where insect food can be more consistently available, due to the thermal inertia of water. *Myotis macropus*, is the only Australian bat to possess enlarged hind feet, capable of trawling water surfaces to glean prey (Churchill 2009), and consequently the only bat exclusively tied to riparian ecosystems (Law & Anderson 1999; Anderson, Law & Tidemann 2006; Campbell 2009; Campbell *et al*. 2009). *Myotis macropus* is also unique in that it is known to have more than one breeding season a year, which is unusual for temperate bat species (Dwyer 1970a). Breeding embodies the highest energetic demands faced by mammals, with lactating females experiencing an 80 % increase in metabolisable energy intake (Korine, Speakman & Arad 2004). It remains unclear how *M. macropus* conserves energy required to meet these metabolic requirements. Thus, investigating torpor use in this bat species offers additional insight and new information on winter torpor use in a mild climate and the influence of a riparian habitat on winter energetics.

My study aimed to measure patterns of thermoregulatory behaviour during winter for *M. macropus*. I used radio telemetry to measure the skin temperature of roosting bats to infer

torpor bout duration, timing of arousals and duration of interbout arousal periods. Furthermore, I investigated how these characteristics were influenced by ambient air temperature, internal roost temperature, sex and the body condition index of individuals. It was predicted that like other similar sized insectivorous bats, *M. macropus* would: i) Use torpor every day when roosting, ii) Prolong torpor bout duration when ambient temperature was colder, iii) Individuals with a lower body condition index would spend more time torpid as they have less energy reserves for rewarming.

3.2 Methods: Temperature telemetry

Study area

The study was conducted from 26/06/2019 to the 1/08/2019 along the Nepean River approximately 50 km west of the Sydney CBD, New South Wales, Australia. Tagged bats roosted in two locations: The Yarramundi bridge on Springwood Road (-33.613, 150.610), and 4 km downstream at the North Richmond bridge on Bells Line of Road (-33.585, 150.724). Yarramundi bridge is a 120 m long, concrete structure, located in the Yarramundi Reserve and surrounded by riparian vegetation and farmlands. Bats roosted in drainage crevices built along the length of the bridge. The North Richmond bridge is 148 m long, made of concrete, and surrounded by riparian vegetation, parklands and urban development. At North Richmond, bats roosted in deep rectangular drainage holes, located periodically under the bridge.

The local area has a humid, subtropical climate, with long-term average daily minimum and maximum temperatures ranging from 3.5 to 17.7 °C in July (winter) at the nearby Richmond RAAF base weather station (1993 to current; Australian Bureau of Meteorology). The greater Sydney region experienced a dryer and warmer than average winter with average daily minimum and maximum temperatures ranging from 3.4 to 19.4 °C. Local external air temperature was taken from automated weather stations at the site of the EucFACE experiment (Ellsworth *et al.* 2017), which is located at the Hawkesbury Institute for the Environment, Western Sydney University Hawkesbury campus (-33.617, 150.740). This site was < 4 km from both roost bridges.

Experimental design

The skin temperature of small bats measured by external radio transmitters can be used to accurately reflect internal body temperature of small bats, with a less than 3 °C error margin (Barclay *et al*. 1994). It has been determined that radio transmitters that are 5 % or less than the total body mass of the bat are acceptable to be used in ethical research (Brigham 1988;Withey *et al*. 2001). *Myotis macropus* weighs 8 to 10 g (Churchill 2009). The transmitters (LB – 2XT, Holohil) used in my study weighed 0.32 g, representing 2.6 % of the total body mass for the species. Only adult bats were included in the study. Transmitters were attached during 5 trapping episodes such that up to 12 bats (6 female, 6 male) were tracked at a time over a 9 to 10-week period (Figure 1).

Trapping bats and attaching transmitters

Bats were trapped at a location approximately 120 m downstream from a colony of *M. macropus* roosting in the Yarramundi Bridge. A single, continuously monitored net (Length; 18 m, mesh size; 16 mm; Ecotone) was deployed across a narrow section of the Nepean River shortly before sunset. Captured bats were removed immediately from the net. An echo meter touch (Echo Meter Touch 2 PRO, Wildlife Acoustics) was used to detect activity of *M. macropus* near the mist net, and a light used to check the net for possibly captured bats.

Trapped bats were placed in small calico bags suspended along a string within a plastic container until processing later that night. The species, maturity (juvenile or adult, gauged by the degree of joint calcification) and sex of each bat was recorded following Churchill (2009). Additionally, the body mass and forearm length of each bat was recorded. For bats to be fitted with transmitters, fur between the shoulder blades was removed using small electric clippers. This assisted with the attachment of transmitters and meant the temperature sensitive area of the transmitter was in direct contact with the skin of bats. A non-toxic latex-based skin adhesive (SAUER skin adhesive, SAUER) was thinly smeared to a small transmitter-sized area of the skin and the surface of the transmitter, and after allowing 30 seconds for the glue to partially set, the transmitter was placed onto the skin using gentle pressure between the shoulder blades of bats. The bat was held for a further five minutes and then released. All bat handling was performed by trained personnel, wearing gloves and vaccinated against Bat Lyssavirus. For all transmitters (LB – 2XT, Holohil) a 2^{nd} order polynomial equation was fitted to calibration data supplied by the manufacturer on pulse interval in milliseconds at 5 °C increments between 5 and 40 °C. Pulse interval was converted to temperature using the specific calibration equation.

Recording data from radio transmitters

The morning following the attachment of transmitters, a hand-held radio receiver (R410, Advanced Telemetry Systems) attached to a three-element antenna was used to determine the location of tagged bats. Data receiver and logger stations (R4500, Advanced telemetry Systems and custom built by Dr G. Körtner) were set up near the location of roosting bats to record the pulse interval for each transmitter. The receivers were programmed to switch on every 10 minutes and cycle through the different transmitter frequencies, and for each frequency detected, record the time interval between two pulses.

Every morning the position of bats was determined using the hand-held receiver. The data was downloaded from the loggers, batteries replaced if need be, and antenna positions

adjusted if bats had shifted and fallen out of range. There were some difficulties with data receivers experienced in the first round of telemetry, resulting in some data loss. Furthermore, the North Richmond bridge roost was longer and larger than the Yarramundi bridge, so at times the radio transmitter signal was too weak for the data receiver to measure the pulse interval.

Data analysis

Bats were considered torpid when skin temperature was < 28 °C (Stawski & Geiser 2010) for more than 10 minutes. However, in some cases the trace of skin temperature indicated that a bat was arousing from torpor but only the initial part of the arousal was recorded, and data was not recorded after warming to around 25 °C. Following these apparent arousals, the trace of skin temperature was recorded again showing a cooling pattern as the bat re-entered into torpor. These partially recorded arousals were counted as arousals from torpor. Occasionally data loggers recorded apparently false measurements of pulse intervals that presumably were caused by interference or a weak signal from the transmitter. These false data were either distinctly beyond physiological limits (the apparent rate for heating or cooling of skin temperature exceeded 1 °C/min)(Burbank & Young1934; Audet & Fenton 1988;Turbill, Kortner & Geiser 2003) or were multiples of two or more times the actual pulse interval. The former data were removed, but the latter were transformed to a single pulse interval by dividing by a numeral (up to 4) so that the transformed values clearly matched with the surrounding data trace.

Once data collection was completed, the following was quantified for each bat:

1) Arousal times; defined as the time of the first skin measurement > 28 °C
2) Start times of torpor bout; defined as the time of the first skin measurement < 28 °C
3) Duration of torpor bouts; defined as the time between the first skin measurement < 28 °C till the first measurement > 28 °C
4) Duration of interbout periods; defined as time between periods of torpor
5) Time away from the roost; defined as the time from the last recorded skin temperature before the bat left the roost till the first recorded skin temperature when it returned.
6) Normothermic period: defined as the time the bat was present in the roost and recorded at temperatures > 28 °C
7) minimum and maximum skin temperature during each bout

8) maximum rate of warming and cooling (°C/min), determined by the change in temperature between measurements and divided by the ten min interval.
9) The body condition index for each bat was calculated as the partial residual from a linear model fitting body mass as a function of forearm length.

Data analyses were conducted using the software *R* version 3.4.2 (R Development Core Team 2009) as implemented by the software RStudio version 1.1.423 (RStudio Team 2015). Mixed effects (LME) models were fitted by restricted maximum likelihood method to test the fit of various models explaining variation in torpor bout duration or interbout duration using the function lmer in the R package lme4 (Bates & Maechler 2009). Individual identity was included in models as random effect on the intercept to account for individual differences or characteristic. Significance of fixed effects was tested using *p*-values and degrees of freedom for the t-test based on the Satterthwaite approximation as implemented by the lmerTest package (Kuznetsova *et al*. 2017). A generalised linear mixed effects model (GLME) was used to calculate the probability of evening arousal. Boot strapping was used, with 500 iterations, to more accurately predict the confidence intervals for the GLME models. Models were simplified from an initial global model by the sequential removal of non-significant fixed effects, starting with least significant interactions. Partial effects plots, which display the model-predicted mean effect, confidence intervals and partial residuals associated with a single variable statistically independent of the mean effects of other variables included in the same model, were derived using the function visreg in the package of the same name (Breheny & Burchett 2017).

3.3 Results

Radio telemetry was conducted from the 26th of May to the 1st of August 2019. The recorded ambient air temperature ranged from 1.3 to 23.6 °C, whereas the internal roost temperature within the concrete bridge was less variable and ranged from 6.9 to 15.4 °C (Figure 1). Mean daily maximum and minimum ambient air temperature over the study period was 18.1 ± 2.6 °C and 6.2 ± 2.7 °C, respectively; whereas the mean daily maximum and minimum roost temperature was 12.8 ± 1.1 °C and 10.5 ± 1.4 °C, respectively.

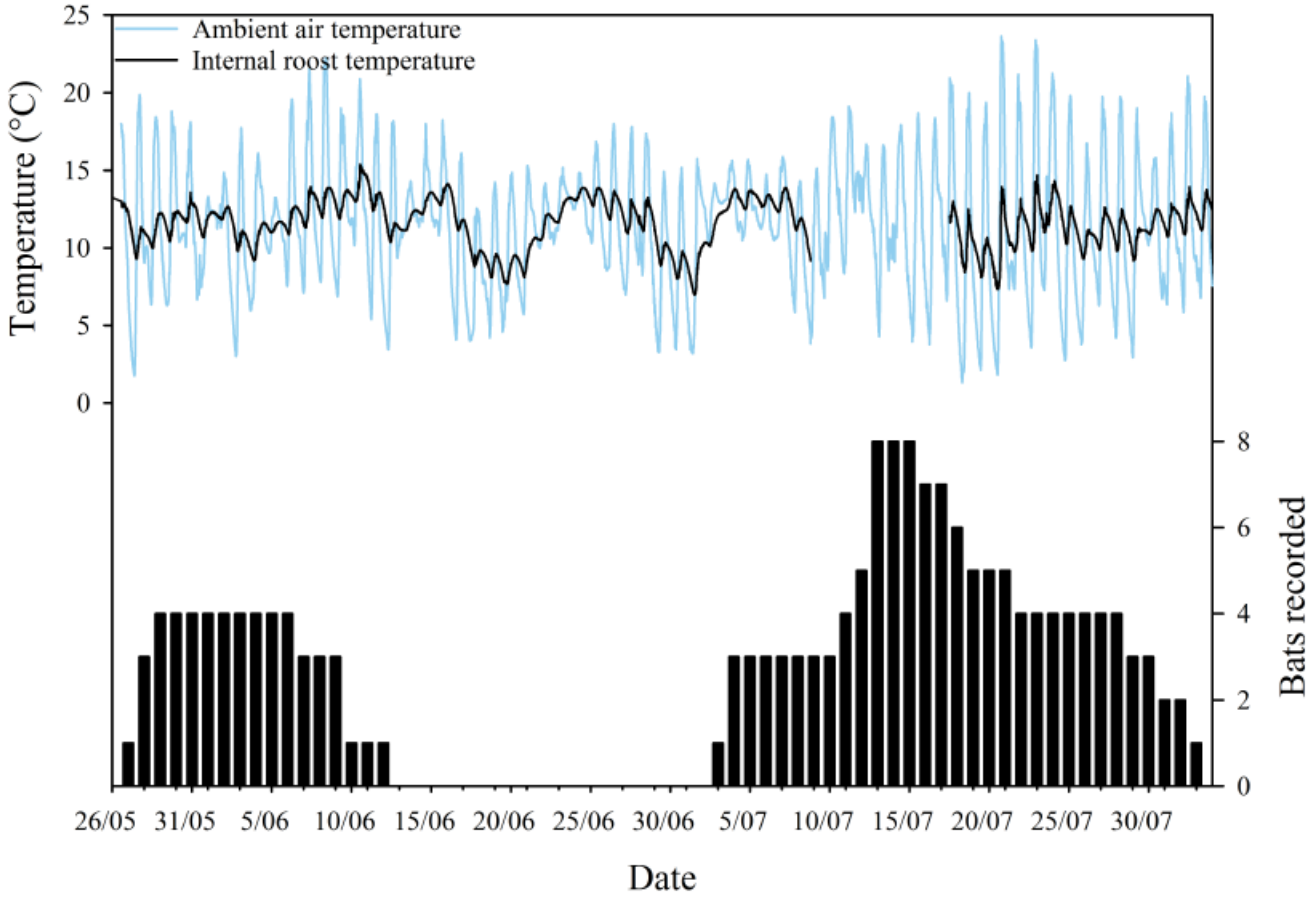

Figure 1. Ambient air temperature (blue line) and internal roost temperature (back line) over the study period (Austral winter). Bars shows the number of bats with recorded skin temperature data for each day of the study period. Roost air temperature data is missing for a period when the logger fell out of the roost crevice.

Skin temperature was recorded over a mean period of 13.4 ± 5.4 days from a total of 12 bats (6 M, 6 F) resulting in a total of 161 recorded bat-days (Figure 2). Tagged bats had a body mass of 10.35 ± 0.29 g for females and 9.93 ± 0.48 g for males and a forearm length of 40.05 ± 0.70 mm for females and 39.18 ± 0.47 mm for males. This equated to a body

condition index of 0.06 ± 0.26 for females and -0.06 ±0.45 for males. However, body condition index of bats was not significant in explaining any torpor bout characteristics modelled in this study.

Throughout the study all tagged bats returned to one of the two roosting bridges, Yarramundi Bridge or North Richmond Bridge. Bats were never recorded moving between bridges, *ie.* a tagged bat roosting at Yarramundi was never located in the North Richmond bridge. Most tagged bats roosted at Yarramundi (n=10), and the remainder roosted at North Richmond (n=2). There was a total of 5 out of 166 bat-days when individuals could not be located at either roost. In these cases, the area around Yarramundi Reserve (approximately 1.4 km^2) and access points (eg., Devlin Road, Windsor Bridge, Howe Park) along the Nepean river were searched, however the bats were never located. It was observed that individual bats returned to similar locations within the bridge throughout the study.

Bats used torpor for the majority of every day recorded and exhibited three typical patterns of torpor use (Figure 2). Most often, bats aroused each day at a similar time close after sunset, remained normothermic for only a brief period, before re-entering torpor (Figure 2a). Sometimes, bats did not arouse in the evening and remained torpid over two or more days (Figure 2b & 2c), lasting a maximum of 6 days.

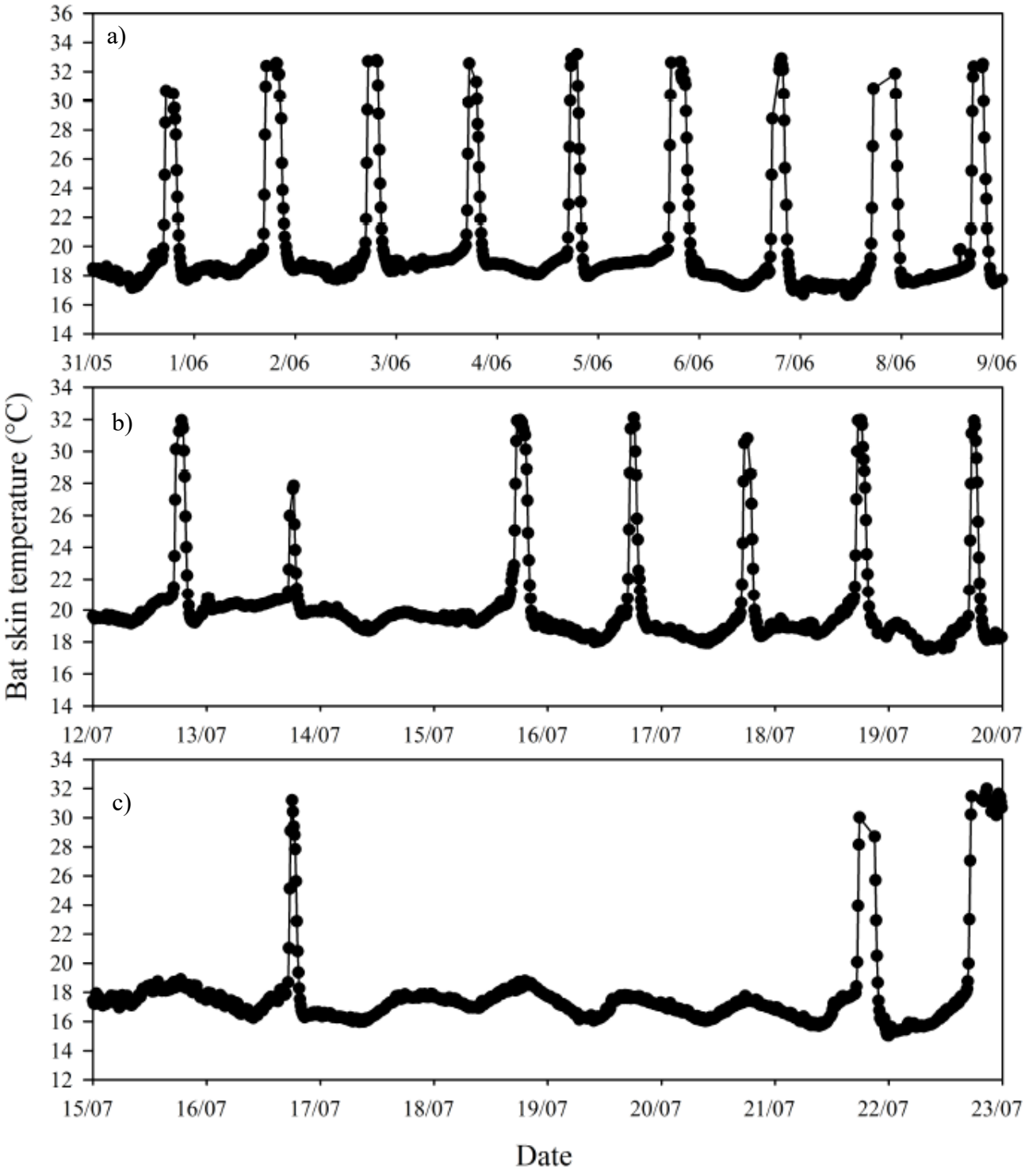

Figure 2. Examples showing three common patterns of torpor use by Australian Fishing Bats at Yarramundi Bridge roost during the Austral winter. Data points are skin temperature recorded every ten minutes. Panels are from different bats and on different dates. Most commonly, bats aroused each evening shortly after sunset (a), but multi-day bouts were also common (b) , and occasionally extended for prolonged periods (c).

The majority (89 %) of arousals took place between 17:00 and 18:00, within an hour of sunset, which occurred from 16:50 to 17:15 during the study (Figure 3).

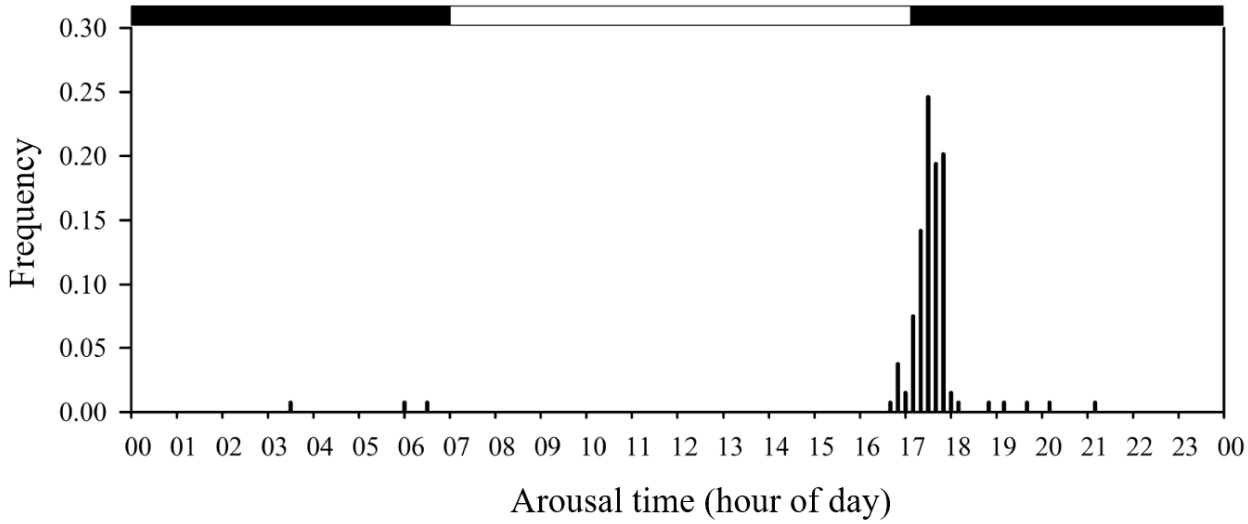

Figure 3. Frequency of recorded times of arousal for all bats (bin width = 10 minutes) over the day. Black bars above the plot indicate the period from sunset to sunrise. Number of observations = 134, from 12 bats.

The median torpor bout duration was 21.3 h, with almost all bouts last around 21 h and some bouts lasting multiples of 24 h, so that the average length of 26.7 ± 21.2 h. Females had a greater proportion of bouts lasting > 24 h, including the longest torpor bout recorded of 144.6 h (Figure 4). A linear mixed effects model fitted to explain torpor bout duration (log_{10}) as a function of sex, which included a random effect of individual identity, found that female sex had a significant, small positive effect on torpor duration (Table 1; Figure 5). The average maximum rate of rewarming of all bats in the study was 0.8 ± 0.11 °C per minute, whereas the average maximum rate of cooling was 0.5 ± 0.08 °C per minute.

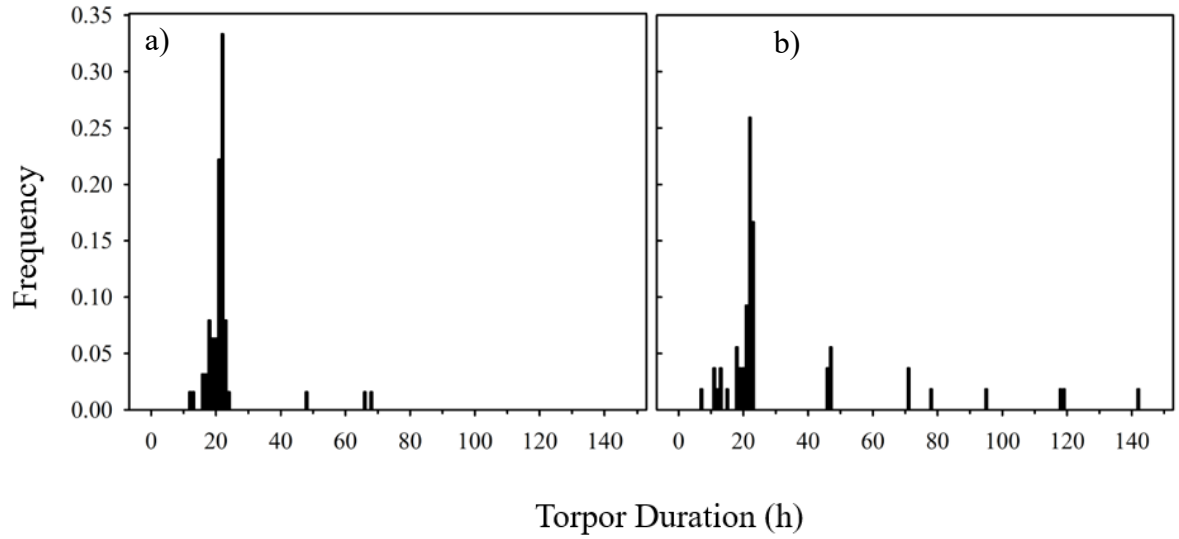

Figure 4. Frequency of the duration of recorded torpor bouts for a) male bats (n=6), number of observations = 63, b) female bats (n=6), number of observations = 54.

Table 1. Results of a linear mixed effects model fitted to explain variation in torpor bout duration (log_{10}) as a function of sex of the bat. Individual identity was treated as a random effect on the intercept. Body condition index was tested and removed from the global model as it was not significant.

Fixed effects	**Coeff**	**SE**	**DF**	***t*-value**	***p*- value**
(Intercept)	1.405	0.0	115.0	50.5	<0.001
Sex (male)	-0.080	0.0	115.0	-2.1	0.038

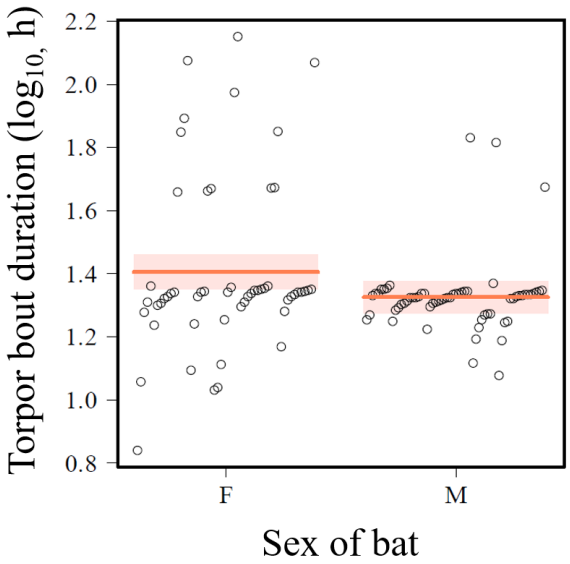

Figure 5. Partial residual plot showing the predicted effect (line) and 95% confidence intervals (shaded) of sex from the linear mixed effects model fitted to explain the log_{10} of torpor bout duration.

A GLME model found the probability of arousal (using only arousals occurring around sunset) was more likely for male bats and on days with a higher daily maximum air temperature (Table 2; Figure 6).

Table 2. Results of a generalised linear mixed effects model fitted to explain the probability of arousal at sunset as a function of the daily maximum ambient air temperature and sex of the bat. Individual identity was treated as a random effect. Body condition index was tested and removed from the global model as it was not significant.

Fixed effects	**Coeff**	**SE**	**z value**	**Pr(>|z|)**
(Intercept)	-2.289	0.005	-465.140	<0.001
Max. air temperature	0.164	0.005	34.340	<0.001
Sex (male)	2.062	0.005	418.930	<0.001

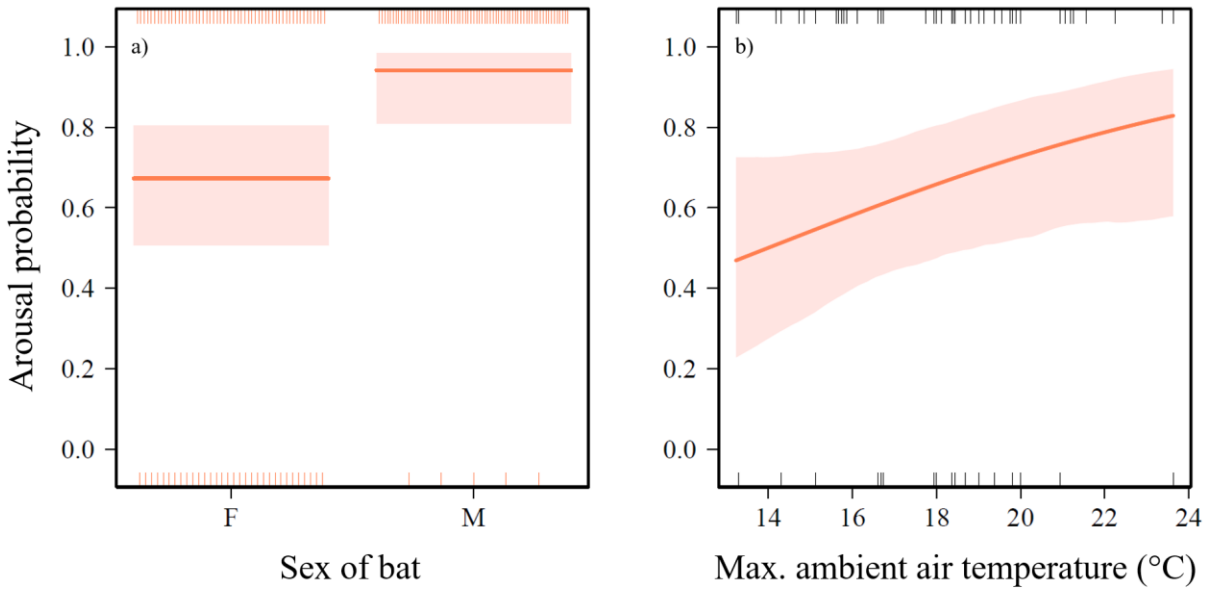

Figure 6. Partial residual plot showing the predicted effect (line) and 95% confidence intervals (shaded) of a) sex of the bat and b) max. air temperature, from the generalised linear mixed effects model fitted to explain arousal probability.

The duration of interbout arousal periods, measured as the time between the end and start of a torpor bout, was 3.5 ± 2.9 h, with a median of 2.7 h. Within these interbout arousal periods, the time when bats were recorded to be normothermic at their roost site (i.e. in reception range of the receiver/logger stations) was 1.1 ± 1.4 h, with a median of 0.7 h. During the remaining portion of the interbout arousal periods, bats were out of range of reception of loggers at both the Yarramundi or North Richmond Bridges. A linear mixed effects model fitted to explain the variation in duration of interbout arousal periods ($\log_{10}$) indicated a positive effect of daily maximum ambient air temperature and male sex (Table 3; Figure 7).

Another LME fitted to explain variation in the duration of time spent normothermic (log_{10}) in the roost indicated only a positive effect of maximum internal roost temperature (Table 3). Ambient air temperature was not included in this model at was highly correlated with internal roost temperature but considered less relevant than roost temperature (see Figure 1). BCI and sex of bats were tested in the global model but removed as they were not significant.

Table 3. Results of linear mixed effects models fitted to explain interbout arousal time (log_{10}) and time spent normothermic(log_{10}) within the roost. Interbout arousal was firstly fitted as a function of maximum ambient air temperature, then sex. Time spent normothermic was fitted as a function of maximum internal roost temperature. Individual identity was treated as a random effect. Body condition index and sex were tested and removed from the global models as they were not significant.

Model	Fixed effects	Coeff	SE	df	*t*-value	*p*-value
Inter bout arousal ~ max. air temp	(Intercept)	0.039	0.2	113.5	-0.2	0.846
	Max. air temperature	0.027	0.0	118.1	2.6	0.012
Inter bout arousal ~ sex of bat	(Intercept)	0.350	0.1	9.8	6.6	<0.001
	Sex (male)	0.173	0.1	8.7	2.3	0.046
Time normothermic ~ max. roost temp	(Intercept)	1.735	0.4	92.7	-4.1	<0.001
	Max. roost temperature	0.122	0.0	86.9	3.8	<0.001

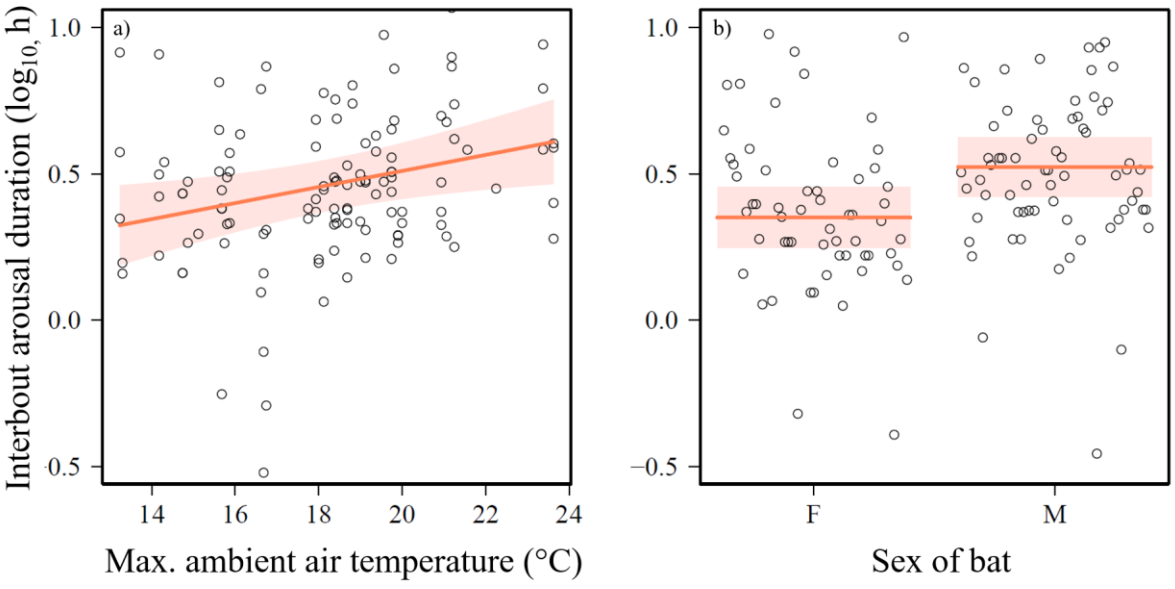

Figure 7. Partial residual plot showing the predicted effect (line) and 95% confidence intervals (shaded) of a) max. ambient air temperature and b) sex of the bat from the linear mixed effects model fitted to explain interbout arousal duration (log_{10}).

3.4 Discussion

This study found that Australia's only fishing bat relies heavily on torpor for energy conservation during winter even in a relatively mild climate. All bats used torpor for the majority of every day and arousal times were brief, especially when maximum ambient air temperature was lower. Sometimes bats remained torpid for several days at a time lasting up till 144.66 h. The probability of evening arousal was lower in colder weather and for females. This study adds to growing evidence that some bat species employ torpor extensively during winter even in subtropical climates (Turbill 2006b; Turbill 2008; Stawski, Turbill & Geiser 2009). *M. macropus* in this study used torpor substantially during winter even though they live in a mild climate and potentially have available food due to their unique ability to trawl for aquatic prey. This indicates the tight energy budgets of the species and small bats in general. Results also suggest that torpor is seasonally driven and could be used to minimise costs in energy and predation associated with leaving safe roosting conditions at a time when activity is not necessary for reproduction or growth.

This study documents the extensive use of torpor by Australia's only fishing bat, *M. macropus*, at a subtropical location during winter. Torpor use by vespertilionid bats is common and helps to reduce resting energy expenditure, evaporative water loss and predation risks (Stawski Willis & Geiser 2014). Thus, it was expected that *M. macropus* would use some torpor, but this had never been tested before and the extent of their winter reliance on torpor was unknown. All recorded bats in my study used torpor every day. Typically bats aroused once each evening, around 17:00-18:00 and torpor bouts most frequently lasted around 22 h. *M. macropus* arousal patterns reflects the brief periods of activity at dusk by free ranging bats in Armidale, Australia, which experiences a slighter colder winter (0.3 to 12.2 °C in mid-winter) than *M. macropus* in my study (Turbill 2008). Torpor bouts were generally shorter than the winter activity patterns found in cold climates, which are usually defined by extended torpor bouts, lasting over several days, with only brief arousal periods (French 1985). However, there were a few instances in which *M. macropus* had multiday torpor bouts, the longest lasting 144.66 h. A similar pattern of torpor use, daily torpor bouts that occasionally extended over several days, was seen by Stawski, Turbill and Geiser (2009) during a subtropical winter. The marine fishing bat, *Myotis vivesi*, also exhibited daily and occasionally multi-day torpor bouts during a desert winter (Salinas *et al*. 2014). Results indicate that torpor use is frequent and significant in the winter behaviour of *M. macropus*.

Myotis macropus was less likely to arouse in the evening when ambient air temperature was lower. Similarly, bats remained torpid for longer than one day when ambient air temperature was significantly lower in a mild climate winter (Stawski, Turbill & Geiser 2009). And torpor duration by bats in a laboratory, with a control temperature of 10 °C, was negatively associated with ambient temperature (Ben-Hamo *et al*. 2013). However, for marine fishing bats strong winds that prevented foraging over water were a stronger predictor of remaining torpid over- night (Salinas *et al*. 2014). In the present study, torpor bouts lasted most of the day with only one, short evening arousal, regardless of changes in ambient air temperature. This suggests season could be a stronger predictor of patterns of topor use. Season significantly influenced torpor duration in two marsupial species, with a 60 % increase in torpor length recorded over winter (Turner & Geiser 2017). And ambient temperature did not affect patterns of summer torpor use by *Nyctophilus bifax* in a sub-tropical study (Stawski & Geiser 2010). However, as radio tracking on *M. macropus* was limited only to winter (and didn't span over the entire season), further study is required for seasonal comparisons of torpor use by *M. macropus* and to determine the drivers of torpor duration.

Warmer ambient air temperature increased the duration of the interbout arousal periods by *M. macropus* during winter. A similar occurrence was recorded by *Nyctophilus bifax* during subtropical winter (Stawski, Turbill & Geiser 2009). Many studies have positively associated bat activity with warmer ambient temperatures (Bender & Hartman 2015; O'Donnell 2000; Russo & Jones 2003), especially when ambient temperature was warm enough to facilitate insect activity (Hope & Jones 2013). A study radio tracking *Myotis evotis* reported that bats foraged all night regardless of changes in ambient temperature (Chruszcz 1999), however, this study was performed during summer when insects are typically more consistently available. The impact of ambient temperature could be more significant in winter for mild climate bats as slight increases in temperature could result in brief winter availability of prey. In my study *M. macropus* spent more time normothermic within the roost when internal roost temperature was higher. Food consumption significantly prolonged normothermic times by Little Brown Bats, *Myotis lucifugus,* with meal size, rather than ambient temperature, being a predictor of torpor bout duration (Matheson, Campbell & Willis 2010). It is possible *M. macropus* spent a greater time normothermic, both outside and inside the roost in response to brief periods of winter foraging.

Female *M. macropus* bats in my study were larger than male bats and the average body condition index of female bats (0.06 ± 0.26) was higher than that of males (-0.06 ± 0.45). This is typical as vespertilionid bats exhibit sexual dimorphism in size with females being the larger sex (Myers 1978; Williams & Findley 1979; Birch 1995). Bats also exhibit sexual differences in activity rates, depending on season and reproductive stage (Banks, Brooks & Schnell 1975). Female *M. macropus* bats remained torpid longer than male bats, were less likely to arouse each evening and had shorter interbout arousal periods. The longest recorded torpor bout was by a female bat, which was also found in another study on torpor use by bats in a mild winter (Turbill 2006b). A telemetry study during subtropical winter found that despite females being larger torpor patterns were similar between sexes (Stawski, Turbill & Geiser 2009). Comparatively Little Brown Bats, *Myotis lucifugus*, hibernating in a cold climate (Canada) winter displayed no effect of sex on hibernation use (Jonasson & Willis 2012). Female *M. macropus* were not pregnant or lactating during the study, thus faced no pregnancy related restriction on torpor use (Grinevitch, Holroyd & Barclay 1995; Stawski 2010) and possibly spent more time torpid to conserve energy for the upcoming breeding season (early spring; September/November) (Dwyer 1970a). Comparatively, increased activity in male *M. macropus* could be associated with the unusual harem roosting dynamics of the species. Dominant males roost with groups of females and adult males often show scars from ear biting, presumable from defending preferred roosts and female groups (Dwyer 1970b). Perhaps the timing of my study meant that over winter competition for female groups had already begun, thus explaining higher rates of activity in males, whilst females were in a rest phase.

Arousal time was very consistent throughout the study, likely coinciding with peaks in insect activity. Eighty-nine % (119/134) of arousals took place between 17:00 and 18:00. Bats typically re-entered torpor after a short interbout arousal and remained torpid till the following evening, similar to the patterns displayed by Australian Long-eared Bats (*Nyctophilus*) in a laboratory study (Gieser and Brigham 2000). Bats maintained this pattern even after being torpid for several days. Arousal time was generally within the hour after sunset, like other bat species (Johnson *et al*. 2012). Rewarming from torpor and increased bat activity is seen to coincide with times that maximise foraging success, enabling animals to gain the most from periods of activity (Hope & Jones 2013; Turner & Geiser 2017). Insect abundance typically peaks at sunset, so the arousal timing recorded by *M. macropus* in this study is generally synchronised with this. Bats can emerge slightly after peaks in evening

insect activity when conditions were darker to avoid higher predation risks (Rydell, Entwistle & Racey 1996). Earlier emergence has been seen by bats with critical energy reserves, suggesting increased risk taking in response to increasing metabolic stress (Duvergé *et al*. 2000). The consistency displayed by radio tracked *M. macropus* indicates strong behavioural trends for torpor use.

The energy expenditure from arousing accounts for approximately 85 % of a bat's winter energy loss (Ben-Hamo *et al*. 2013) and activity comes with inherent risks of predation as well (Turbill & Stojanovski 2018). Therefore, there must be an underlying motivation to drive daily arousals by both males and females. The interbout arousal periods by *M. macropus* were typically very short, so bats may not have been rewarming for foraging purposes. Patterns of brief evening arousals has been seen in other bat species during mild winter conditions (Turbill 2006b; Turbill 2008). Rewarming can take place for hydration after evaporative water loss during torpor (Willis *et al*. 2011; Ben-Hamo *et al*. 2013). Additionally, torpor negatively impacts immune system efficiency so rewarming can occur in order to initiate immune response to potential pathogens (Burton & Reichman1999; Luis & Hudson 2006). Returning to a high normothermic temperature can also aid digestion and waste removal (Speakman & Rowland 1999). It is thought that winter rewarming by male bats is associated with copulation (Turbill 2008), however this is unlikely to be the case for *M. macropus* as they mate during early spring (September/November) unlike other bat species that mate at the beginning of winter and store sperm or postpone offspring development (Dwyer 1970a). There are benefits to rewarming even during rest phases for short periods if energy reserves or prey are available to support this.

Body condition index had no influence on torpor use in winter by *M. macropus*. A higher body condition index resulted in deeper and longer torpor bats by *Nyctophilus bifax* in sub-tropical summer (Stawski & Geiser 2010). Similarly, fatter mouse lemurs, *Microcebus murinus*, had deeper torpor bouts than leaner lemurs in a mild climate dry season (Vuarin *et al*. 2013). However, there was no influence of BCI on the hibernation patterns of Little Brown Bats, a similar sized insectivorous bat in North America (Jonasson & Willis 2012). Chruszcz (1999) proposed that *Myotis evotis* were using torpor at times that were not considered an energy emergency. Furthermore, in a laboratory study conducted under mild temperatures (7 and 17 °C) bats did not use torpor to its full energy saving potential but varied patterns to keep a balance between energy input and output (Matheson, Campbell &

Willis 2010). Body condition might not strongly influence torpor use given bats are using torpor regardless of a critical need.

Throughout the study *M. macropus* were located roosting at Yarramundi Bridge and North Richmond Bridge. The majority of bats (n=10) roosted at Yarramundi bridge, and individuals were never recorded moving between bridges. Furthermore, individuals of both sexes appeared to favour the same location in the bridge for roosting, returning to the same area repeatedly. However, there were five days for two bats when roosts could not be located at either bridge or in surrounding areas. In a study on the roosting dynamics of *M. macropus* by Dwyer (1970b), female bats were not rigid in roost selection, roosting in small groups but interchangeable within these groups. Males were much more likely to return to the same roosting spots typically alone or possibly in small groups of other males. Similarly, throughout this study it was observed that there were several groups of bats roosting in colonies within the bridge as well as singular bats roosting separately, assumedly males.

In conclusion, my study documented the first evidence of torpor use by *M. macropus*. Ambient air temperature and sex both significantly affected torpor bout duration and the probability of evening arousal. The metabolism and energy budgeting of small mammals is complex and the factors effecting these critical physiological processes are not fully comprehended. My study shows that torpor is used extensively even in mild climates and by riparian bats, that likely have prey available more consistently over winter. Results add to the growing literature that torpor is a wide spread adaption, useful to small mammals across a variety of climates and habitat types.

Chapter 4: General conclusion

My thesis studied the influence of day- to- day changes in weather (especially temperature) and season on the activity of insectivorous bats and insects, how these relationships changed with habitat type and, when accounting for environmental conditions, how are bat and insect activity patterns related. Both bat activity and insect abundance were positively associated with temperature, with a remaining influence of season. However, the strength of these relationship was significantly different between bat species and insect orders or size classes, and there was a weaker influence of temperature in riparian compared to woodland habitats. For some bat species, specifically the riparian specialist *Myotis macropus*, there was no significant relationship between activity and temperature. However, season did strongly influence the activity of this species. Bat activity had a stronger positive relationship to insect activity only when air temperature was relatively cool, which suggests bats are active in cold conditions if there is available prey to support energetic demands. Appropriately timing periods of rest and energy conservation are critical for small mammals. Measuring the skin temperature of roosting *M. macropus* showed they employed frequent and prolonged torpor during winter. Temperature and sex significantly affected torpor use, with male bats and higher maximum temperatures invoking higher probability of evening arousal.

The results of my study have identified a number of knowledge gaps that require future research. These include:

- Seasonal changes in piscivory of the diet of *M. macropus*, and how this might relate to their activity.
- By what mechanism(s) can insectivorous bats detect increased insect activity in cold conditions, and how do they time their activity to maximise the benefits of this opportunities.
- Seasonal changes in torpor use by *M. macropus*, and how torpor use varies between sexes depending on their reproductive condition (females: pregnant, lactating; males: social status and harem position).
- In a rapidly changing climate, photoperiod may no longer accurately reflect environmental conditions and timing of prey availability. Research could attempt to understand if such mismatches are affecting *M. macropus*.

The ability of animals to appropriately schedule periods of activity and rest is critical for their survival and is a crucial part of the ecology of small endothermic animals feeding on temperature-dependent food sources. Small endotherms have high energy needs and it is important that they maximise the benefits of activity, which is why activity typically coincides with peak prey abundance (Hope & Jones 2013; Turner & Geiser 2017). By understanding the intricacies of ecosystem function and the effect of environmental conditions on activity patterns we may advance the field of ecology and manage habitats for wildlife conservation.

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